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Meniscal ossification in spontaneous osteoarthritis in the guinea-pig

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Summary

Objective: The purpose of this study was to evaluate the ossification state of the meniscus in the guinea-pig stifle joint using micro-computerized tomography.

Design: Hind limbs from six (*N*=12) and 24 (*N*=11) month-old male Hartley guinea-pigs were removed and the joints were imaged using high resolution micro-computerized tomography. The ossified volume of the medial and lateral menisci from both groups of animals was quantified.

Results: Ossification of both the medial and lateral menisci of the both the 6- and 24-month-old animals was observed. In both age goups, the ossified region of the medial meniscus was significantly larger than the lateral meniscus. In addition, there is a significant increase in ossified volume of the medial meniscus between 6 and 24 months of age.

Conclusions: There is a significant amount of ossification of the menisci in the male Hartley guinea-pig, with the medial compartment showing more bone than the lateral. In addition, as the animals age, there is an increase in ossification within the medial compartment. Bone remodeling and cartilage degeneration is evident in the medial compartment within these animals as they age. It is possible that the increased ossification of the medial meniscus could alter the joint biomechanics and, in part, stimulate this medial compartment joint destruction. © 2000 OsteoArthritis Research Society International

Key words: Osteoarthritis, Guinea-pig, Micro-CT, Bone, Cartilage, Meniscus.

Introduction

Age-related spontaneous osteoarthritis (OA) in the guinea-pig was first described over 40 years ago.[1] Since then, it has been studied using enzymatic[2] as well as histological techniques.[3,5,7–9] Classical hallmarks of human OA[10] such as cartilage proteoglycan loss, chondrocyte cell death and cloning, cartilage swelling and fissuring, subchondral bone scelerosis, cyst and osteophyte formation have been observed in this model.[1,3,5,7,8] A limited number of micro-CT (μCT) and *in vivo* MR imaging[11–13] studies have reported alterations in bone architecture and increased cartilage swelling, respectively, in this animal model. Degenerative changes (proteoglycan loss and cloning)[5] of the menisci of the stifle joint have also been reported; quantitation of the volume of ossified tissue within the menisici has not been reported. The goal of this study was to assess the age-related changes and to measure the volumes of the ossified region of the menisci (ORM) in the guinea-pig stifle joint using 3-D μCT.

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Materials and methods

Male Hartley guinea-pigs were housed and cared for in accordance with the procedures using lab animals as approved by the Institutional Animal Care and Use Committee of SmithKline Beecham Pharmaceuticals. Six-month-old (*N*=12) and 24-month-old (*N*=11) guinea-pigs were killed and their hind limbs removed and fixed in 70% ethanol. The patellae were removed and some of the soft tissue around the stifle joints was trimmed to fit the samples in the sample holder for μCT imaging. Images of the intact right joints were obtained on a μCT-20 scanner (Scanco Medical, Bassersdorf, Switzerland). A total of 334 slices (30 μm thick) with an in-plane resolution of 30×30 μm and an integration time of 75 ms/projection, yielding a total imaging time of 4.5 h, were collected from each joint. The raw images were gauss-filtered (sigma=1.2, base=2) and binarized using a constant threshold for all the samples and rendered for 3-D display. The volumes of the ORM were calculated from unprocessed images using *ANALYZE* (Mayo Clinic, Rochester, MN, U.S.A.) image processing software. A mixed model split-plot analyses of variance was used to test the differences in the 6-month- and 24-month-old animals as well as the differences between the lateral and medial side. The mixed model analyses accounted for the interdependence between the lateral and medial positional

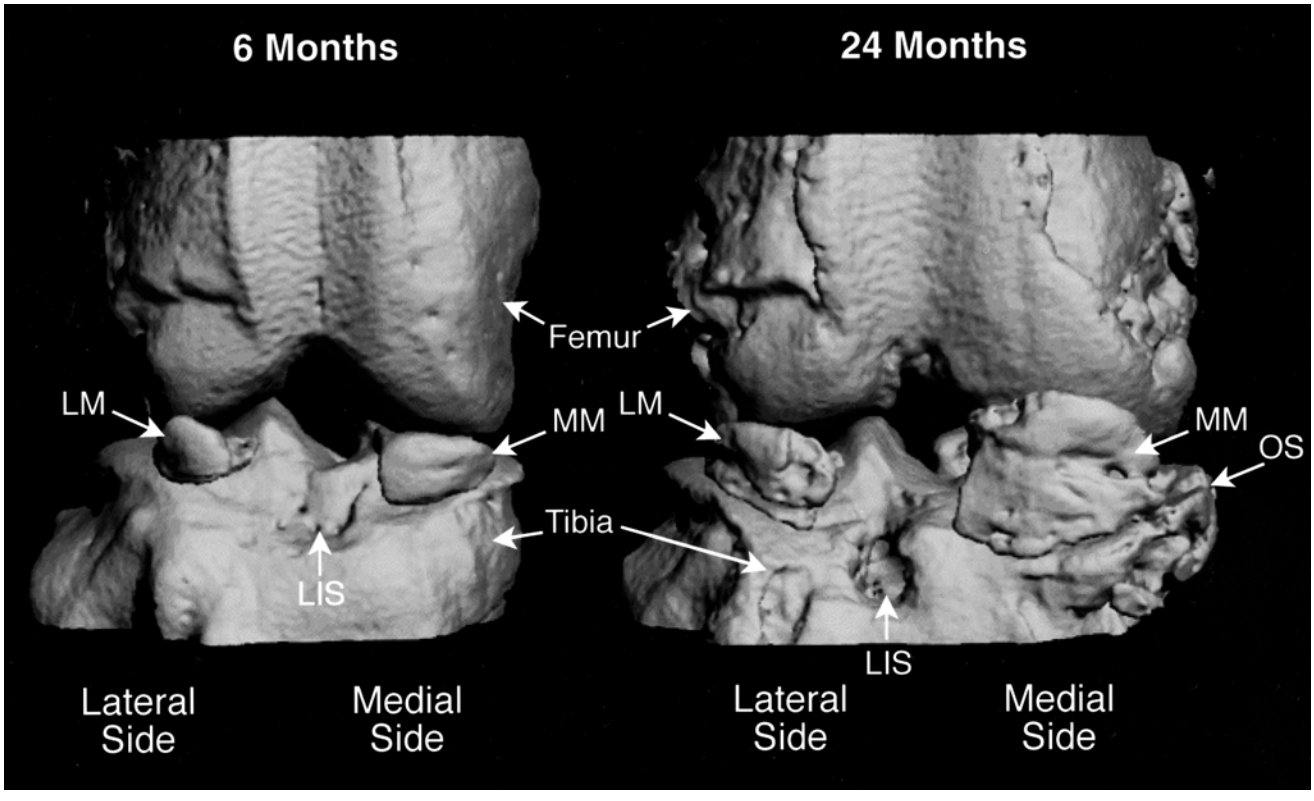

Fig. 1. Anterior view of representative 3-D micro-CT images of the stifle joint of a 6-month-old and a 24-month-old guinea-pig obtained at a resolution of 30×30×30 μm (LM: lateral meniscus; MM: medial meniscus; OS: osteophyte; LIS: ligament insertion site).

data from the same animal. In order to reduce the chance of false positives, the estimated differences from the mixed models were compared to a simulated multivariate *t*-distribution critical values for significance. All statistical analyses were done at the 5% significance level.

For histological evaluation, frontal paraffin sections through the cruciate ligament attachments of the tibia were collected and stained with toluidine blue (0.1% in 0.1 M acetate buffer, pH 6.2). Normal cartilage matrix stains dark blue (indicative of a high concentration of matrix proteoglycan), whereas degenerating OA cartilage stains pale blue (indicative of proteoglycan loss).

Results

Representative images of the anterior views of 3-D rendered μCT images of the stifle joints from a 6-month- and a 24-month-old guinea-pig are shown in Fig. 1. The image of the joint from the 6-month-old guinea-pig shows normal joint architecture and normal bone surfaces on the tibia and femur. The ligament insertion site (LIS) on the tibia can be visualized. There does not appear to be osteophyte formation on the tibial plateau at this age. The images also demonstrate the meniscal ossification in both the medial and lateral compartments. There is a significant difference in the volume of the ossified region between the medial and the lateral sides in the 6-month-old guinea-pig (P=0.003) group.

The image of the 24-month-old guinea-pig shows marked osteoarthritic changes including subchondral bone surface changes on the tibia as well as the femur, cyst-like formation at the site of ligament insertion and osteophyte formation on both the medial and the lateral sides. Dispersed ossified regions, removed from the bone structures, can be visualized and could be attributed to calcification in ligaments and/or tendons. There is a significant difference in the volume of the ossified regions of the meniscus between the medial and the lateral side (P<0.001) at 24 months. In addition, there is an increase in the ossified region of the meniscus (ORM) volume on the medial side of the joint between the 6- and 24-month-old groups (P=0.005). The ORM volume on the lateral side of the joint shows a similar increase but did not reach statistical significance (P=0.401). The results are summarized in Fig. 2. It should be noted that there also appears to be some ossification occurring in the posterior part of the menisci (lateral or medial) in about half of the joints at this age.

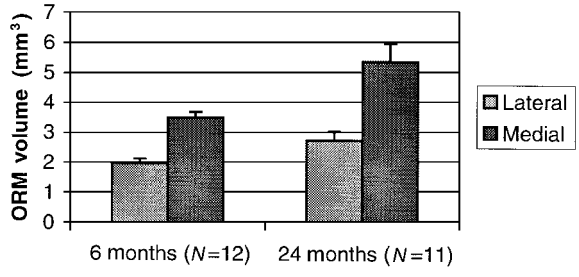

Fig. 2. Ossified region of the menisci volumes (lateral and medial) of the guinea-pigs at 6 months and 24 months. Significant differences between lateral and medial ORM volumes at the two ages (P=0.003; 6 months and P<0.001; 24 months) can be seen as well as an age-related increase in the medial (P< 0.001) ORM volumes.

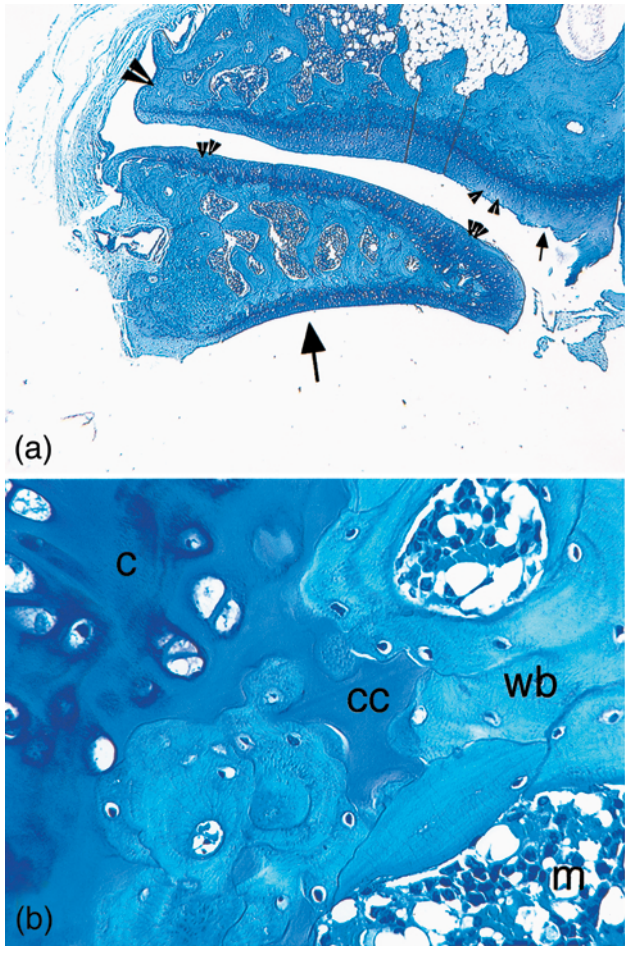

Fig. 3. Bone formation within the medial meniscus of a 24-month-old guinea-pig. (a) Low power view of the medial meniscus (large arrow) that covers the medial tibial plateau (top). The central zone of cartilage has been replaced by bone (light blue). Note the degenerating cartilage (small arrow), reduced toluidine blue staining of the cartilage matrix (small arrowheads; indicative of proteoglycan loss), and the large peripheral osteophyte (large arrowhead), within the underlying medial tibial plateau. Proteoglycan loss and matrix degeneration is also apparent in the meniscal cartilage (small double arrowheads). Magnification ×10. (b) High power view of (a) demonstrates woven bone (wb) forming on the eroded spicules of calcified cartilage (cc). The peripheral cartilage (c) and marrow space (m) are indicated. Magnification ×100. Paraffin section stained with Toluidine blue.

Histological evaluation revealed that the bone forms within the central region of the menisci, and is histologically equivalent to a developing secondary ossification center that develops into the long bone epiphysis (Fig. 3). Thus calcification of the central zone of the meniscal cartilage is followed by vascularization and resorption of the calcified cartilage. Osteoblasts subsequently form layers of woven bone onto the residual cartilage spicules. The final histological appearance at 24 months is similar to an articular surface, with underlying subchondral bone, trabecular bone and marrow space [Fig. 3(a)].

Discussion

While there have been numerous studies examining articular cartilage and subchondral bone changes in the guinea-pig OA model,[1–9,11–13] quantitation of meniscal ossification and the possible role it plays in development and progression of OA have not been reported. In both the animal model and the human disease, there is dramatic cartilage degeneration, chondrocyte cloning, osteophyte formation, synovial hypertrophy, and meniscal degradation. Osteoarthritis in humans can result from a number of factors including genetic predisposition, joint trauma or joint disuse.[10] It appears that the disease develops and progresses due to some type of altered joint biomechanics. This could result from joint trauma resulting in ligament or meniscal tears, obesity resulting in significant loading of the joints, work-related joint stress or congenital defects resulting in altered joint architecture. In the guinea-pig, the age-related increase in volume of the ossified region in the medial meniscus could result from dramatically different loading forces in the medial versus lateral compartment due to the bowlegged posture of the animal.[8] This bone within the meniscus could then contribute to a further load imbalance within the joint and possibly stimulate significant metabolic changes within the articular cartilage. Altered loading of cartilage has been shown to cause increased matrix degradation as well as increased chondrocyte cell death.[14] The converse relationship between manipulation of load distribution either by osteotomy[6] or by reducing weight[4] and degree of cartilage damage in the guinea-pig has also been demonstrated.

In addition to being a model of OA, the guinea-pig model may be a model which reflects, in some aspects, human chondrocalcinosis. In chondrocalcinosis, there is mineralization of both the articular cartilage and meniscus, synovial hypertrophy and cartilage thinning.[15,16] In addition, a larger proportion of patients with this condition have classical OA than the total population.[17] It is possible that the increased mineralization observed in humans with chondrocalcinosis could result in part to altered joint biomechanics, ultimately resulting in cartilage destruction.

The unambiguous volumetric measurements of the ossified regions of the menisci may offer an index for monitoring the *in vivo* progression of OA as well as the effects of therapeutics in this animal model of human OA. Further work to characterize this phenomenon and its role in the development of OA in the guinea-pig is in progress and could offer novel insights to the role of articular meniscus in the development of OA in humans as well.

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