Quantitative ex Vivo MR Histology in Experimental Dog Osteoarthritis

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Introduction

Conventional preclinical drug research in the area of osteoarthritis (OA) involves final assessment of cartilage lesions with gross morphology (visual inspection) and histology (microscopic evaluation) end-points. Both these methods are mostly qualitative, subjective, labor-intensive, and prone to tissue processing artifacts. We present the use of high resolution ex vivo MR imaging of dog knee joints as a quantitative end-point and a "ground truth" assessment of cartilage morphology for use in preclinical OA drug research.

Methods

The in vivo portion of experiment was approved by appropriate local IACUC. Eighteen mixed breed female hounds (28-32 kg) were subjected to the surgery (Anterior Crucial Ligament tear, ACLt, N = 9, and sham N = 9). Twelve weeks after the surgery animals were euthanized and their both operated and contralateral knee joints extracted and fixed in the formalin for at least 48 hours. Joints were then disarticulated and placed in the sealed containers in the air or Fomblin media for imaging. MRI was performed on a 7T Bruker Biospec scanner equipped with 12 cm ID gradient insert (up to 20 G/cm) and 72 mm ID birdcage volume RF coil. Optimal slice planning was performed using information obtained from quick orthogonal images in the coronal, axial and sagittal aspects. High resolution 3D GRE images were acquired in the sagittal aspect with the following acquisition parameters: TE = 2.4 ms, TR = 25 ms, FA = 30º, resolution of 125 × 125 × 400 µm. For high SNR imaging was performed overnight with acquisition time up to 13 hours/bone. Final images were randomized and cartilages (femoral and tibial, lateral and medial) were segmented using an automatic classification approach with subsequent minor manual correction. The bone-to-cartilage and cartilage-to-air/Fomblin contrasts were very high allowing easy and mostly automatic segmentation of the cartilage. The area, volume, and average thickness of cartilage were computed. After MRI knee joints were subjected to conventional gross morphological and histological analysis.

Results

The representative MR image of ACLt opereated dog's femur is shown in Figure 1A. Figure 1B depicts the corresponding histology slice of the same subject. The cartilage was segmented successfully in all images. Figure 2 shows the comparison of femoral cartilage volume measurements for both groups of animals including the non-operated leg. ACLt operation led to overall increase in the volume and average thickness (not shown) in femoral cartilage compared to Sham group and contralateral leg control. Despite this general increase of cartilage volume and thickness in ACLt subjects they demonstrate localized areas with decreased thickness (see 3D rendering of cartilage in Figure 3), which are consistent with the classical understanding of cartilage lesion development during OA.

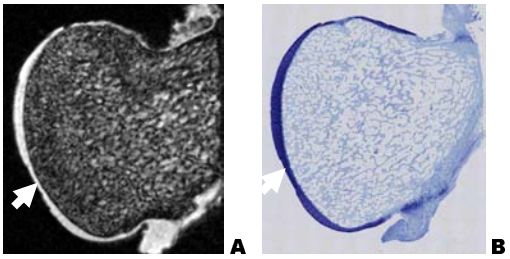


Figure 1. Representative ex vivo MRI image of dog's femur (A) and corresponding histological slide (B). Note the similar appearance of cartilage legion (arrow).

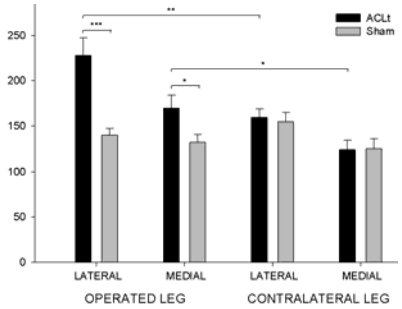
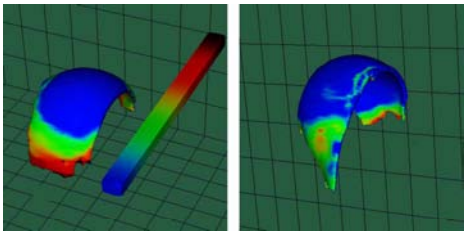


Figure 2. Comparison of femoral cartilage total volume (mm^3) between ACLt and Sham animals and between operated and contralateral leg. Data are Mean ± SEM. *, **, *** - significant differences between groups: P < 0.05, 0.01, and 0.001 respectively

Discussion

One of the challenges of validation of existing methods of quantization of in vivo cartilage morphology in OA is the absence of absolute reference, the so-called "ground truth" data. Both gross morphology and microscopic histology methods could only provide relative visual correlation with apparent lesion and deriving quantitative parameters from these methods would require substantial effort and time investment. In this study we propose to use ex vivo high resolution high contrast MRI as a surrogate end-point reference to validate the in vivo MRI for preclinical OA studies. Although this method can not provide a true "ground truth" data due to inevitable shrinkage of the tissue after fixation, but it could add substantial information to validate in vivo imaging and to investigate cartilage behavior in preclinical models of OA. Further improvement of quantization methods and sensitive local lesion detection algorithms is required to provide better understanding of cartilage involvement in the complex development of OA and to advance drug research and development in this area.



SHAM ACLt

Figure 3. 3D rendering of femoral lateral cartilage from ACLt and Sham operated dogs. The color bar on the left panel encodes the thickness from 0 (black) to 0.75 mm (blue).