Quantification of age dependent molecular changes in guinea pig OA model using T1ρ MRI

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Background:

Osteoarthritis (OA) is a degenerative joint disease, which causes severe pain and is associated with compromised quality of life and imparts heavy toll on the health care system. Knee and hip OA are especially important because they are primarily the OA cases contributing to chronic disability amongst the elderly population [(1)]. More than 50% of U.S. residents over 65 years of age experience pain and limitation in mobility due to knee OA[(2)]. Currently there is no cure for the disease and therapeutic interventions are primarily targeted to symptomatic relief. Various animals have been shown to develop OA [(3)] either spontaneously or through surgical methods. Age dependent biochemical analysis of the Dunkin-Hartley guinea pig stifle has been studied extensively demonstrating spontaneous OA progression [(4)]. The purpose of this study was to demonstrate the efficacy of $T_{1\rho}$ MRI in quantifying age-dependent, spontaneous osteoarthritic changes in the Dunkin-Hartley guinea pig model over a period of 10 months and correlate these data with histopathology.

Materials and Methods:

All experiments were performed with approval from the Institutional Animal Care and Use Committee. Images were obtained from the guinea pig left stifle joint using a four-shot T1ρ prepared 3D balanced gradient echo sequence (bGRE) on a 9.4T horizontal bore animal scanner (Varian). Three female cohorts were selected (3-4 month {N=8}, 5-6 month {N=6}, 9-10 month {N=5}) for this study. Animals were euthanized immediately after imaging and were not used for further age cohorts. T1ρ MRI was performed with the following parameters: Slab: 30 mm × 30 mm× 8 mm, Matrix: 512 × 256 × 16, Interpolated resolution: 58 μm × 58 μm × 500 μm, flip angle: 20º, TE: 4.7 ms, TR: 9.2 ms, centric k-space encoding, Averages: 6 per shot. A four-shot acquisition was used to mitigate loss of T1ρ-contrast as a steady-state magnetization was reached. A thermal equilibrium delay of 3.5 seconds was added to the end of the segments to allow full growth of the longitudinal magnetization. The spin-lock amplitude was fixed at 1500 Hz for all scans. Five T1ρ-weighted images (spin-lock durations: 1, 10, 20, 30, 40 ms) were acquired for each animal. Raw k-space data were filtered to mitigate blurring due to transient signal decay as described previously [(5)]. Images were then co-registered to a high-resolution bGRE image. Images were fitted on a pixel-by-pixel analysis to the linearized, mono-exponential signal decay equation to generate T1ρ relaxation maps. Mid-coronal slices of the stifle were used to segment cartilage into four compartments (medial/lateral tibial/femoral) using the SliceOMatic (Tomovision, Quebec, CA). Segmented masks were then used to report mean T1ρ values for each compartment.

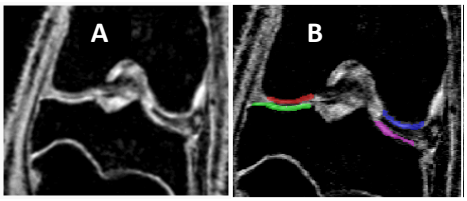

Figure 1 – bGRE image used for segmentation (A) with masks overlaid (B)

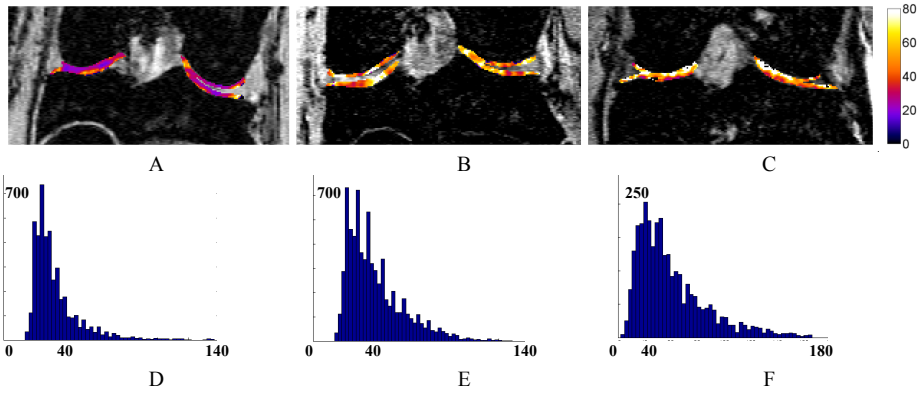

Figure 2 - Representative mid-coronal images with T1ρ maps and associated T1ρ histogram - 3 month (A-D) 5 month (B-E) and 9 month (C-F). Color bar in ms. Horizontal: T1ρ value, Vertical: pixel count

At the time of this abstract, complete biochemical analysis of all joint tissues have not been completed. Tests measuring cartilage content and damage by histology, metachromatic dyes, and immunohistochemistry will be performed. Each stifle imaged will be dissected, decalcified, and processed for paraffin embedding. Assessments will be made for overall integrity of the cartilage using sections stained for proteoglycan content (Alcian blue and Safranin-O) and overall ECM characteristics (Masson's Trichrome). Grading will be performed using the Mankin score and quantification of specific stains for proteoglycan will be made using image analysis software (Image-Pro Plus, MediaCybernetics, Bethesda, MD). Values for proteoglycan content measured will be tested for correlations with T1ρ data.

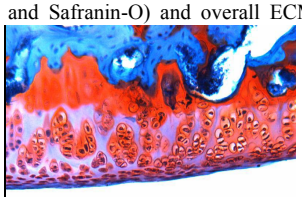
Figure 3 – Representative 3 month old stained with Safranin O, specific for proteoglycan (red).

Results:

Figure 1 shows a typical high-resolution bGRE image (1A) used for segmentation with overlaid segmented masks (1B). Figure 2 shows representative T1ρ maps from a 3-4 month (2A), 5-6 month (2B), and 9-10 month (2C) old guinea pigs. A one-tailed Student's t-test was performed to determine differences in T1ρ values between age cohorts. In the analysis, data was averaged for all four zones for each sample. A *P* value <0.00001 was found between the 3-4 month cohort and 5-6 month cohort. A *P* value <0.001 was found between the 3-4 month and 9-10 month cohorts. A non-significant *P* value (0.27) was found between the 5-6 and 9-10 month old cohorts. Significant cartilage damage and thinning was observed in 9-10 month old animals compared the younger cohorts. Analysis of the skewness of the T1ρ distribution shows a *P* value <0.0005 between the 3-4 month cohort and the 5-6 month cohort and between the 3-4 month cohort and the 9-10 month cohort. Routine histology (Safranin O) to detect proteoglycan content was performed on select guinea pigs and representative histological section of a 3-4 month old stifle is shown in Figure 3. These results are consistent with those reported [(4)].

Discussion:

There is a 39% increase in mean T1ρ values between the 3-4 month and 5-6 month old cohorts and a 45% increase between 3-4 month and 9-10 month old cohorts. Between 5 and 9 month old animals, the lack of significant difference of T1ρ values indicates that significant cartilage matrix molecular degradation has already occurred by month 5-6. In 9-10 month old cohort, while the $T_{1\rho}$ values are higher than those in younger cohorts, visible cartilage damage, fibrillation, and thinning has occurred. Additionally, T1ρ distribution becomes significantly skewed towards high T1ρ values in the two older cohorts. The histological characterization, assessments of cartilage integrity, and the quantification of changes in proteoglycan composition in the cartilage of all animals, will determine when OA can be detected ahead of late stage morphological changes. Collectively, this will provide the needed data to support the use of this sensitive imaging method to measure biochemical changes in articular cartilage during the early stages of joint diseases such as OA and provide a non-invasive and non-destructive method to rapidly evaluate the efficacy of potential disease modifying therapies in animal models.

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