Response of Quantitative MRI to Artificial Collagen Cross-linking of Articular Cartilage

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TARGET AUDIENCE: Researchers aiming to apply quantitative MRI techniques in osteoarthritis research.

PURPOSE: Aging of cartilage results in accumulation of advanced glycation end products (AGEs) in cartilage, some most important of which are collagen crosslinks. Increased cross-linking of the collagens makes the cartilage matrix more stiff and brittle [1]. The aims of the study were to evaluate the sensitivity of various quantitative MRI parameters (T1, dGEMRIC, T2, continous wave (CW) T1ρ, adiabatic T1ρ, adiabatic T2ρ and magnetization transfer (MT)) to artificial collagen cross-linking induced by L-threose (threose) in bovine articular cartilage [2].

METHODS: Osteochondral samples (n = 7 + 7) of 6 mm were prepared from bovine patellae obtained from local slaughterhouse. The specimens were incubated for 6 days 18 h, half of the specimens were treated with threose (Sigma Aldrich Co., St. Louis,MO, USA) while the other half served as controls. After the treatment the specimens were frozen at -20°C until the experiment. Contrast enhanced computed tomography studies on the adjacent tissue of the specimens have been reported earlier [2]. MRI was performed at 9.4 T (Oxford instruments Plc, Witney, UK) using a 19-mm quadrature RF volume transceiver (RAPID Biomedical GmbH, Rimpar, Germany) and Varian DirectDrive console (Varian Inc. Palo Alto, CA, USA). Prior to imaging, the specimens were thawed, placed inside a Teflon test tube and immersed in perfluoropolyether with the cartilage surfaces perpendicular to B_0. Quantitative MRI measurements were carried out by utilizing a magnetization preparation (MP) block or spin-echo preparation placed prior to the FSE readout, or by using saturation recovery technique. Measured parameters included adiabatic T1ρ (HS1 pulses, pulse duration 4.5 ms, γB_{1max} = 2.5 kHz), adiabatic T2ρ (HS1 pulses, pulse duration 4.5 ms, γB_{1max} = 2.5 kHz), CW-T1ρ with spin-lock powers γB_1 = 125 and 1000 Hz, and T1 during saturation (T1sat) at +10 kHz off-resonance (MT experiment), T1 and T2 relaxation times and dGEMRIC measurement after 24h Gd-DTPA^{2-} immersion.

For determination of biomechanical properties of the cartilage, equilibrium modulus (E_{eq}) was measured using a step-wise stress-relaxation test (3x5% step, 100%/s strain rate). The fixed charge density (FCD) content of cartilage and collagen fibril anisotropy (parallelism index, PI) and orientation were quantified with digital densitometry and polarized light microscopy from Safranin-O and unstained sections, respectively. Furthermore, the water content of the cartilage was determined from the samples as the ratio of dry and wet weights. The different cross-links in cartilage (hydroxyl pyridinoline (HP), lysyl pyridinoline (LP) and pentosidine (Pent)) were quantified using high performance liquid chromatography and the collagen and proteoglycan (PG) contents were measured with FTIRI.

Full-thickness cartilage ROIs from the relaxation time maps were analyzed for mean and standard deviation (SD) values of threose-treated and non-treated groups. Statistical differences of the MRI parameters between the groups were investigated with Wilcoxon signed rank test.

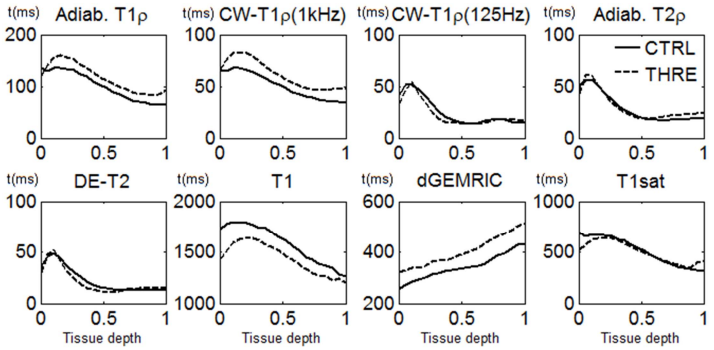

Figure 1. Depth-wise profiles of adiabatic T1ρ, CW-T1ρ at spin-lock powers of 1000 and 125 Hz, adiabatic T2ρ, adiabatic double echo T2, T1, dGEMRIC and T1sat relaxation time parameters in threose treated (THRE) and non-treated (CTRL) cartilage groups.

RESULTS: Cross-linking (Pent and LP), stiffness (E_{eq}) and FCD (optical density) were significantly ($p < 0.05$) increased (18.2 mmol/mol, 77 mmol/,mol, 1.02 MPa and 1.75, respectively) in the threose treated cartilage samples compared to the non-treated samples (<0.004 mmol/mol, <0.032 mmol/,mol, 0.35 MPa and 1.39) [2]. However, PG, collagen and water contents of cartilage as well as the orientation and anisotropy of the collagen fibrils remained unchanged. The dGEMRIC technique showed significantly longer relaxation times in the threose treated samples in comparison with non-treated cartilage while native T1 relaxation time was significantly decreased (Table 1). T2 and T1sat exhibited no statistically significant differences. Of the rotating frame MRI parameters, adiabatic T1ρ and CW-T1ρ (at spin lock frequency of 1 kHz) were significantly increased with the threose treatment while adiabatic T2ρ showed no statistically significant differences. CW-T1ρ demonstrated dispersion and at 125 Hz spin-lock power the relaxation times were close to T2 values and the statistical difference between the groups vanished (Figure 1, Table 1).

Table 1. *Summary of the relaxation times (mean ± SD (ms)) in full-thickness ROI of threose-treated and non-treated cartilage. * p<0.05 Wilcoxon signed rank test*

Parameter (n = 7)	t(ms) threose	t(ms) non-treated
Adiabatic T1ρ	113.1 ± 23.0*	97.8 ± 20.9
Adiabatic T2ρ	31.8 ± 8.5	27.7 ± 3.7
CW-T1ρ, γB_1 = 1.0 kHz	62.7 ± 10.7*	48.9 ± 11.7
CW-T1ρ, γB_1 = 125 Hz	27.4 ± 10.2	23.7 ± 2.5
DE-T2	20.7 ± 5.5	26.4 ± 15.0
T1	1400.4 ± 100.4*	1551.0 ± 105.2
dGEMRIC	454.2 ± 27.3*	357.5 ± 23.2
T1sat	500.1 ± 91.6	490.7 ± 57.9

DISCUSSION: Similarly to the previous contrast agent enhanced CT study [2], threose treated specimens demonstrated decreased penetration of the negatively charged contrast agent due to increase of the FCD, as a result of cross-linking of cartilage. That resulted in elevated relaxation time values with the dGEMRIC technique, even though the PG content remained unchanged. Furthermore, a trend towards shorter T2 values was observed in threose-treated group. In a previous study [3], T2 was significantly decreased due to cross-linking, however the fixation was done in nasal cartilage with formalin, different from the present experiment. Increase of adiabatic T1ρ as well as the CW-T1ρ was an unexpected finding. This observation could be attributed to slower dynamics due to cross linking, which escaped detection at relatively high spin-locking fields, i.e., at 1 kHz of spin-lock power, or blocking of exchange-mediated relaxation. This relaxation phenomenon could be used for evaluation of natural aging of articular, and thus requires further research.

CONCLUSION: Threose-treatment detectably changed the biophysical properties of articular cartilage and provided an original means to the study of relaxation processes in cartilage. Quantitative MRI parameters were sensitive to threose-induced artificial cross-linking in cartilage, similar to natural aging. While dGEMRIC responded to the threose treatment as expected, the T1ρ parameters showed unexpected increase in the relaxation time constants which is possibly attributed to an increased cross-linked fraction characterized by the slow motion or blocking of exchange-mediated relaxation.

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