Assessment of Degradation of Proteoglycans and Matrix Proteins in Intervertebral Disc Degeneration by Diffusion Weighted MRS

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INTRODUCTION: Intervertebral disc degeneration (IVDD) is considered to be the root cause of back pain. It is a common disease of high morbidity in aging population (1-3). Currently, correlation between MRI and CT morphologic findings and patient symptoms in disc degenerative disease was poor (4). Previous high-resolution HR-MAS studies have revealed the relationship between spectroscopy derived metabolite concentrations in 3.5-4.2ppm,namely the carbohydrate (Carb) region and levels of disc degeneration (2,4). However, in clinical MRS study, the quantification of Carb region resonances is technically challenging due to the relatively poor shimming in vivo and broad spectral overlap by water peak (3). In this preliminary study, we hypothesize that diffusion of water is much faster than that of macromolecules which generate the Carb region resonances, thus we propose to suppress the water peak contamination by diffusion weighting. Furthermore, we hypothesize that diffusion characterization of macromolecules resonances including Carb region resonances can provide a sensitive marker for IVDD.

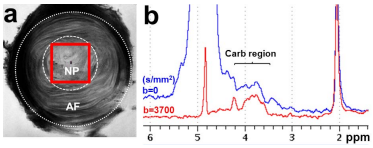

Fig.1 (**a**) Axial T2-weighted image of a normal bovine disc (NP, nucleus pulposus; AF, annulus fibrosus; VOI selection shown as the red rectangle) (**b**) Diffusion weighting (DW) suppresses the large overlapping water contamination and allows the reliable measurement of Carb region (3.5-4.2ppm) resonances in a normal disc.

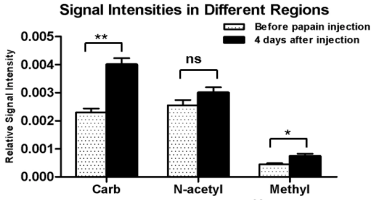

Fig.3 Signal intensities in different regions (N=6, normalized by water signal) before and after papain injection.

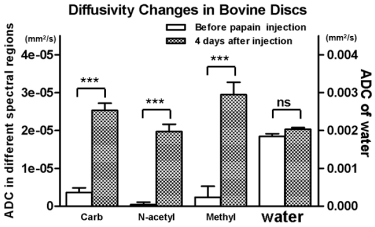

Fig.4 Diffusivity changes in bovine discs before and after papain injection (N=6).

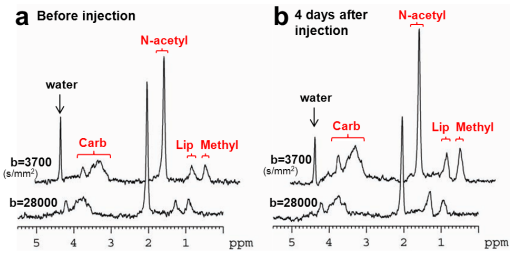

Fig.2 Representative DW spectra acquired from a bovine disc before (**a**) and 4 days after papain injection (**b**). Four major resonances were observed and assigned as Carb region (3.5-4.2ppm): C-H resonances of GAG, ethanoloamine and glycine; N-acetyl region (1.9-2.1ppm): N-acetyl group of GAG, proline and glutamate; Lip region (1.2-1.4ppm): aliphatic chain of lipid and lactate; Methyl region (0.8-1.0ppm): isoleucine, leucine and valine(2,4,5).

MATERIALS AND METHODS: Model Preparation**:** A total of 6 intervertebral discs were harvested from the fresh bovine spine. To induce disc degeneration, each disc was injected with 50uL solution containing: 1mg papain (28U/mg), 0.01M L-Cysteine hydrochloride, 0.01M Ethylene Diamine Tetraacetic Acid (EDTA) and 0.1M sodium phosphate. After injection, all discs were kept at 4^{o}C for 4 days. The DW-MRS measurements were performed before and 4 days after injection. Protocol**:** All DW-MRS experiments were performed on a 7T Bruker scanner. For DW-MRS, a STEAM based single-voxel MRS sequence was implemented by adding a pair of unipolar diffusion gradients in two TE/2 intervals. Diffusion gradient was applied along the main magnetic field. Diffusion weighted spectra were acquired with TR/TE = 1000/25ms, diffusion duration δ=8ms, diffusion time Δ=150ms, 2 b-values (3700 and 28000 s/mm^2). Data Analysis**:** Spectral analysis was performed using JMRUI and TOPSPIN software package. Signals in Carb region (3.5-4.2ppm), N-acetyl region (1.9-2.1ppm) and Methyl region (0.8-1.0ppm) were quantified by fitting the spectrum to multiple peaks of Lorentzian line shape using AMARES algorithm. The signal intensities and apparent diffusion coefficients (ADCs) in three regions were calculated for each disc by fitting the diffusion weighted signals to a mono-exponential decay model. Results were considered significant when p<0. 05. (Two-tailed paired t-test, *p<0.05;**p<0.01;***p<0.001).

RESULTS: Figure 1a shows the DW-MRS voxel location based on the T2-weighted reference image. In each disc, the nucleus pulposus (NP) part of 120μL volume was selected for DW-MRS. The efficient separation of water and Carb region resonances by diffusion weighting was demonstrated in Figure 1b. Before diffusion weighting, the Carb region resonances were buried under the broad water peak. After applying diffusion weighting with appropriate b-value, the water peaks was effectively suppressed/eliminated and the Carb region resonances could be readily detected and quantified. In diffusion weighted spectra, four main spectral regions could be observed (Fig. 2). Four days after papain injection, significant increases were observed in the relative signal intensity in Carb and Methyl regions whereas, in N-Acetyl region, there was also a slight increase though statistically insignificant (Fig. 3). At the same time, the diffusion properties of the three regions were found to change drastically after papain injection. Before injection, ADCs in Carb and Methyl regions were below 10^{-5} mm^2/s while ADC in N-acetyl region was even smaller. However, 4 days after papain injection, ADC values in all these three regions increased by more than ten folds (p<0.0001) (Fig. 4), indicating that these macromolecules now became significantly more mobile. In the same discs, water ADCs were also measured and seen to increase slightly from 1.90±0.11×10^{-3}mm^2/s to 2.03±0.10×10^{-3}mm^2/s after papain injection. Note that this water diffusion increase was comparable to the finding in a previous human study (6).

DISCUSSIONS AND CONCLUSION: Previous biochemistry study indicated that degradation proteoglycans (PGs) of plays a major role in IVDD progression, which is the main cause of disc dehydration and reduction of osmotic pressure in disc matrix (1,7). Papain digestion is a widely used model of disc degeneration in IVDD research because papain is a relatively non-specific protease that can break down both PGs and the disc matrix proteins (3,7). In this preliminary study, the resonances in Carb and Methyl region increased substantially after papain injection, which was in agreement with the earlier HR-MAS findings showing that the number and intensity of free amino acids resonances increased in the degenerated human discs (2,4). A slight signal intensity increase was also observed in N-acetyl region, but not as notable as Carb and Methyl regions. This observation may arise from the fact that the resonance intensity of amino acids (proline, glutamate) in 1.9-2.1 ppm was low, thus difficult to yield statistically significant difference (Fig. 2). More importantly, apparent diffusion coefficients (ADCs) of Carb, N-acetyl and Methyl groups were observed to increase drastically after papain injection, indicating the great potential of these macromolecular diffusion measurements as robust and sensitive makers in detecting disc degeneration. We reason that the byproducts of disc degeneration, including both amino acids and degraded glycosaminoglycans (GAGs), had much smaller molecular weights than PGs and matrix proteins. At the same time, GAGs was known to be highly hydrophilic. Once no longer anchored within the disc matrix, GAGs dissolved into the water, thus exhibiting greatly increased mobility. These factors together led to the drastic ADC increases observed in this study. In conclusion, diffusion weighting provides an effective method to separate and quantify carbohydrate resonances by suppressing the large overlapping water peak. More importantly, DW-MRS provides a new and clinically translatable approach to probe the biophysical processes in IVDD. In particular, MR characterization of carbohydrate, N-acetyl and Methyl macromolecular groups holds great potential to sensitively and quantitatively detect and assess the degradation of PGs and matrix proteins during disc degeneration. Future studies will include the longitudinal examination of papain injection and other disc degeneration models, optimization of DW-MRS sensitivity, and clinical implementation and assessment.

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