Detection of Extracellular Matrix Degradation in Intervertebral Disc Degeneration by Diffusion Magnetic Resonance Spectroscopy (DW-MRS)

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INTRODUCTION: Intervertebral disc degeneration (IVDD) is a leading cause of low back pain. IVDD is a slow process that likely develops over decades (1-3). Proteoglycans (PGs) are the critical component of the extracellular matrix (ECM) in the disc nucleus pulposus (NP). Currently, all existing MR methods target the pathophysiological events that occur after the onset of ECM degradation and PG loss. As a result, clinical IVDD diagnoses are made mostly at relatively late stages. We hypothesize that the breakdown of PGs and collagen in the disc NP during IVDD will lead to increased mobility of macromolecules, and diffusion weighted MRS (DW-MRS) is sensitive to measure these macromolecular mobility increases and detect the ECM degradation processes early and sensitively.

MATERIALS AND METHODS: Intervertebral discs were harvested from the fresh bovine spine and were individually embedded in 5% agarose gel. Papain (1mg) was injected into the NP to induce IVDD using a previously reported procedure (4). After injection, discs were longitudinally examined over 5 days at room temperature and stored at 4°C between scans. Protein gel electrophoresis (SDS-PAGE) was performed right after the MR experiments. All experiments were performed using a Bruker 7T scanner. On each disc, the nucleus pulposus (500μL) was selected for DW-MRS. A STEAM based single-voxel MRS sequence was implemented (TR/TE/TM= 900/34/110ms, Δ/δ=127/10ms, NEX=512). Macromolecular T2 values were measured with a non-DW sequence using different TEs (TR=1500ms, TE=15/30/45/60/75ms). Water ADC and T2 values were measured using multi-echo SE and DW EPI imaging sequences. Spectral analysis was performed using JMRUI. The signals in Carb (3.5-4.2ppm), N-acetyl (1.9-2.1ppm) and Methyl region (0.8-1.0ppm) were quantified by fitting the spectrum to Lorentzian line shape with AMARES algorithm. Relative macromolecular contents were computed and corrected by T2. Water ADC and T2 value were calculated from a circular VOI covering the NP region. Results were considered significant at $p<0.05$. (Two-tailed paired t-test, *$p<0.05$; **$p<0.01$; ***$p<0.001$).

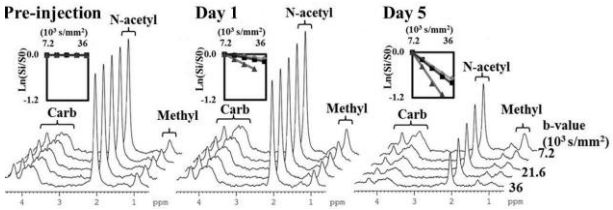

Fig.1 Typical diffusion weighted (DW) spectra and corresponding DW signal decays before and after papain injection. Three major resonances were observed and assigned as: Carb region (C-H resonances of GAG, ethanoloamine, glycine), N-acetyl region (N-acetyl group of GAG, proline, glutamate), Methyl region (isoleucine, leucine, valine).

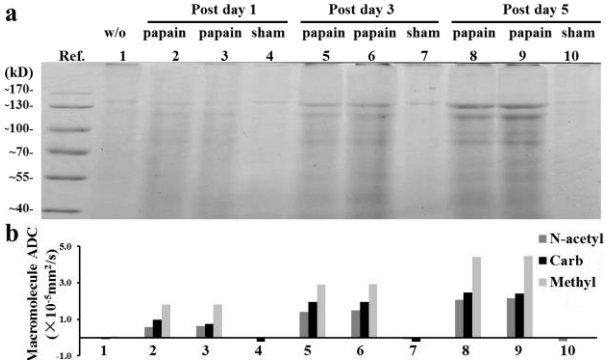

Fig.4 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (**a**) and the macromolecular ADCs **(b)** measured in 10 discs**.**

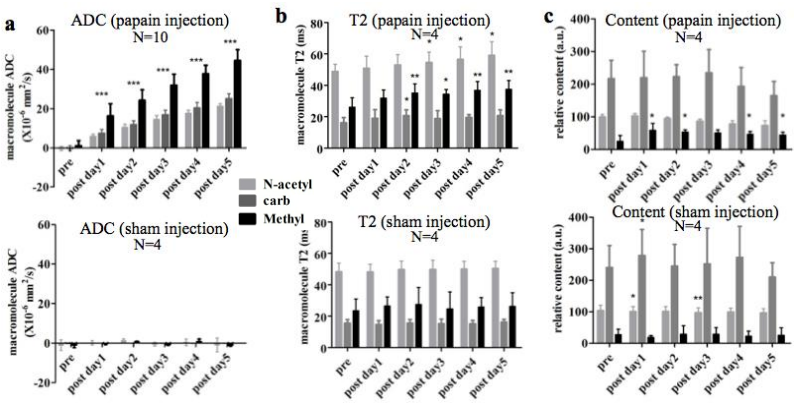

Fig.2 (**a**) Longitudinal macromolecular ADC measurements in discs (N=10) treated with papain and sham-treated control discs (N=4). (**b-c**) Macromolecular T2 values and the relative contents (after T2 correction) measured in papain-treated (N=4) and shame-treated discs (N=4).

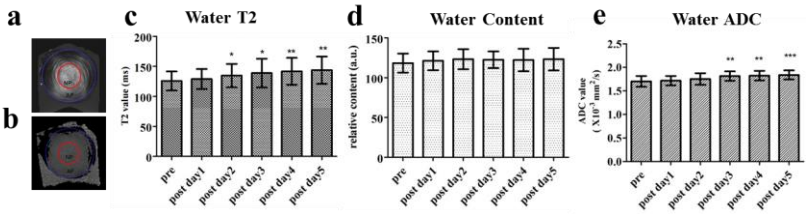

Fig.3 (**a-b**) Representative water T2 and ADC maps with red circle depicting the measurement ROI. (**d-e**) Longitudinal measurements before and after papain injection (N=10).

RESULTS: Typical DW spectra are shown in Fig. 1. DW signal decays in all three regions were observed to increase drastically as early as 1 day after papain injection, indicating that the corresponding macromolecules had become significantly more mobile. Fig. 2 summarizes these macromolecular ADC changes in 10 papain-treated discs and 4 sham-treated discs as controls, together with T2 values and relative contents measured in a subset of 4 papain-treated discs and 4 controls. Papain-treated discs generally exhibited slight increases in T2 values and slight decreases in contents likely due to the increased macromolecular mobility and leakage from the disc, respectively, whereas the sham-treated controls showed virtually no changes. Fig. 3 shows the corresponding water content, T2 and ADC measurements, indicating slight increase in water T2 and ADC, no change in water content. To confirm the ECM degradation, protein gel electrophoresis was performed in both papain-treated and sham-treated discs. Fig. 4 shows that papain-treated discs exhibited gradually increased spread of macromolecular sizes/weights under 170kD and gradually increased macromolecule ADCs, indicating the presence of more and fragmented macromolecules, whereas the control discs showed no obvious change.

DISCUSSIONS AND CONCLUSION: The macromolecule ADC increases most drastically in the methyl region (Fig 2) which may be contributed by the amino acid residues in small peptide fragments of degraded collagens. Those small peptides have relatively smaller molecular weight than fragmented PGs. While for the Carb and N-acetyl region, the resonances are partially from PGs, thus it is understandable that the Methyl resonance had the highest ADC value among the three. Macromolecule T2 also increased during ECM degradation (Fig.2b) that may be due to the mobility increase of the macromolecules (5,6). With the increasing T2 during IVDD, the macromolecule content measurement by conventional MRS method could be compromised. We corrected the macromolecule content by T2 and found the N-acetyl resonance content is gradually decreased 1 day post papain injection, resulting from the gradually leaking of the detached GAG chains. The macromolecule ADC may be more sensitive to the ECM degradation than the N-acetyl resonance content, which represents the PG content in clinical MRS studies (7). Our findings demonstrated that DW-MRS could be used to characterize ECM macromolecule degradation, providing a direct and more sensitive method to detect IVDD. DW-MRS can also provide assessment at molecular and microstructural levels, which is imperative for both clinical and preclinical development of new treatments for IVDD.

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