HR-MAS Spectroscopy of Human Intervertebral Disc Tissue Demonstrates the Lactate/N-Acetyl Ratio as a Potential Marker for Painful Degenerative Disc Disease

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Introduction

The clinical evaluation of low back pain is difficult. The intervertebral disc is thought to be a primary source of this pain. MR is useful for grade disc morphology, but not specific enough to identify painful discs because altered morphology does not necessarily equal painful discs. The current gold standard to determine the specific disc causing back pain is provocative discography and it is invasive, painful and has a positive predictive value of only 50-60%. (1) There is also a lack of objectivity in patient reported pain. There is currently no non-invasive marker for disc pain. The N-acetyl peak of proteoglycans (chondrointin sulfate, keratan sulfate, and heparan sulfate) is resolved at 2.04ppm and has been shown to decrease with disc degeneration. (2) Another published HRMAS disc study suggested that lactate and the proteoglycan N-acetyl levels could provide a quantitative method for identifying painful intervertebral discs. (3) However, this prior study did not compare patients with degenerated discs with and without pain, which is the critical clinical question, and the focus of the current study. To determine whether chemical biomarkers can discriminate painful from non-painful degenerated discs, *ex vivo* long echo-time ^{1}H high-resolution magic angle spinning (HR-MAS) spectroscopy data were acquired from patients with intervertebral disc tissue and correlated with a clinical assessment of pain.

Methods

Twenty-one intervertebral discs were surgically removed from 15 different patients with degenerated painful (N=12) and degenerated non-painful (N=9) diagnoses. The disc nucleus was pathologically separated from the annulus prior to the HR-MAS study. Discogenic pain patients were defined by a combination of pathologic findings on MRI and CT discography. In this abstract, the focus is on the chemical changes in the nuclear samples since in *in vivo* ^{1}H PRESS spectroscopic disc patient studies, the select volume is centered on the nucleus (4). Samples were weighed (mean 15.13 ± 7.98) and placed into custom designed 20 or 35 µl leak proof zirconium rotors containing 3.0 µl D_2O + 0.75% TSP. ^{1}H HR-MAS data were acquired at 11.7T, 1°C, and 2,250 Hz spin rate using a Varian INOVA spectrometer, equipped with a 4 mm gHX nanoprobe. A long echo time rotor synchronized Carr-Purcell-Meiboom-Gill (CPMG) sequence was acquired with 2s relaxation, 2s presaturation, 2s acquisition (TR = 6s), 40,000 points, 20,000 Hz spectral width, 144 echo time and 512 transients. The Electronic Reference To access In vivo Concentrations (ERETIC) (5) method was used as a quantitation standard. A student's T-test was performed to determine if there was a significant difference between the two groups of patients.

Results

Figure 1 shows representative 1D cpmg HR-MAS spectra of a) non-painful degenerative and b) painful degenerative intevetebral discs. T_2 filtered data was used in hopes of resolving the lactate doublet at 1.33ppm from lipids; however, as can be seen in the painful disc spectrum, residual lipid often remained prohibiting the accurate quantification of the lactate doublet in disc samples. However, the lactate quartet was well resolved and of a sufficient signal to noise ratio to robustly quantify lactate in cpmg spectra. Visually, both spectra demonstrate chemical changes typical of disc degeneration, including, an increase in resolution of the resonances in the carbohydrate region of the spectrum, decreased N-acetyl and increased lactate and alanine peaks. Important to the current study, there was a 2-fold higher mean lactate in painful vs. non-painful disc tissue, but due to the large variability of lactate measurements in both patient cohorts, the difference in lactate levels was not significant (4.14±4.72 vs. 2.17±1.11, p=0.19). Additionally the N-acetyl peak was not different between the painful and non-painful cohorts (23.65±27.05 vs. 17.74±8.17, p=0.49). The relative ratios of the lactate/N-acetyl are shown in figure 2 provide the best discrimination of painful from non-painful degenerated discs. The ratio in the painful discs (mean = 0.19 ± 0.08) was significantly higher than in non-painful discs (mean = 0.12 ± 0.03, p=0.02), however there was still overlap of the individual values.

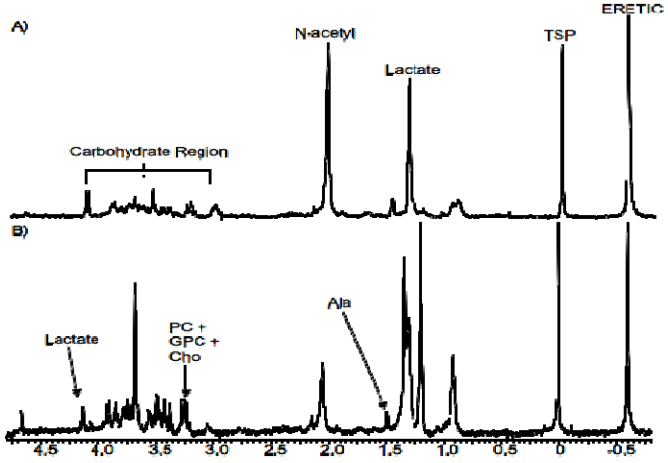

Figure 1: ^{1}H HR-MAS spectra of A) non-painful and B) painful degenerative intervertebral discs.

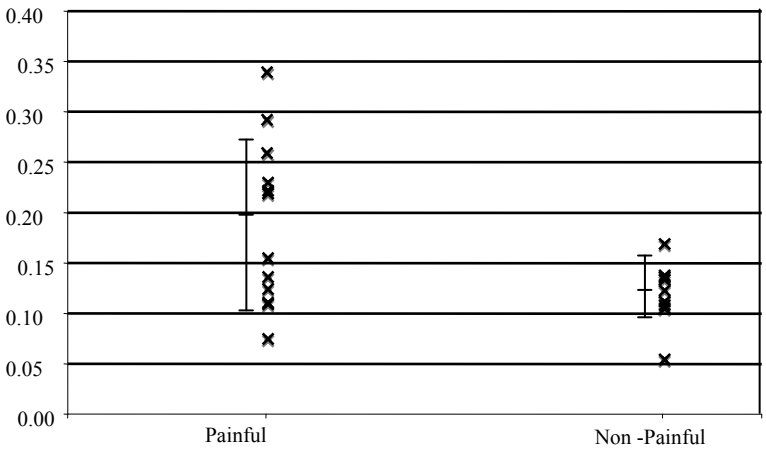

Figure 2: Lactate/N-acetyl ratio in painful and non-painful degenerative intervertebral disc.

Discussion and Conclusions

This study demonstrates that the lactate/N-acetyl ratio has potential for quantitatively discriminating painful from non-painful degenerated discs. However, overlap in the individual lactate/N-acetyl ratios warrants further study to understand whether the overlap is due to a true overlap in disc chemistry or insufficient clinical classification of pain. Additionally, the predictive ability of the lactate/N-acetyl ratio could be improved through the addition of other chemical markers such as water and lipid content as well as other imaging approaches, such as T1ρ.

References

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