INTRODUCTION: Much work has been performed to characterize degenerative disc disease including gross morphologic, biochemical and histologic grading schemes (1,3,4). To better understand and treat disc degeneration, the responsibility has been placed on researchers to develop an accurate and non-invasive diagnostic tool able to objectively detect changes in the matrix with age and degeneration. Quantitative MR allows for an objective and reproducible way of assessing the MR sensitive protons and their interaction with matrix macromolecules. Our group has been able to correlate T1, T2, and magnetization transfer with quantitative MR techniques to disc biochemical content and found that all these MR parameters were intimately linked to both the matrix biochemical makeup and grade of disc degeneration(2). We have also shown that apparent diffusion coefficient (ADC) is not a reflection of the disc’s biochemical subunit content. When the ultrastructural state of the disc is accounted for the apparent diffusion coefficient is a direct reflection of matrix integrity. We have identified that the information obtained with ADC can be used in combination with quantitative T1, T2 and magnetization transfer to give us an accurate assessment of the disc matrix composition and structural integrity. Despite these advances, we do not know to what extent collagen and/or proteoglycan degradation are responsible for changes in quantitative magnetic resonance values. The aim of this study is to induce the changes seen on quantitative MR using directed enzymatic degradation. To this end, we injected various concentrations of collagenase and trypsin in the bovine intervertebral disc while performing quantitative T1, T2, MT and ADC assessments.

METHODS: Twenty-four bovine caudal intervertebral disc were harvested. The discs were injected with buffer solution, 2mg/ml or 5mg/ml of trypsin or collagenase in the nucleus pulposus region. The specimens were allowed to incubate over 18hours. The samples were then attached to six standard solutions of 19.2 and 38.6 mM MnCl2, and 2% and 4% agar. Standard SE sequences were used for T1, T2, and MT determination. ADC was determined along the anterior/posterior axis using an SE sequence (TE 25ms, TR 700ms, TD 150 ms, Diffusion Gradient 0-24 mT/m). Specimens were then isolated from the nucleus pulposus region. These samples were extracted with a 4 M guanidinium chloride solution. The extract material was used to quantify GAG content (µg/mg) using the DMMB (dimethylmethlene blue) dye binding colorimetric assay. Adjacent specimens were dried at 110 °C for 4 days to obtain the dry weight.

RESULTS: ANOVA analysis of GAG content demonstrated a significant drop in GAG content with collagenase and trypsin treatment (p<0.05). Water content was not significantly altered by either collagenase or trypsin treatment. Our results indicate a gradual and significant (p<0.05) drop in T1 with increasing concentrations of collagenase (from 1400ms to 1000ms). A more subtle drop in T1 is noted with increasing concentrations of trypsin - from 1400ms to 1150 ms (p nil signif). T2 values also dropped markedly with increasing concentrations of collagenase - from 230 ms to 130 ms (p<0.05). Once again, the effect noted with trypsin is more subtle- from 210ms to 160 ms (p nil signif). The effect of enzymatic degradation on the magnetization transfer was subtle with no discernable trend being noted in the trypsin treated group. The collagenase treated group demonstrated a trend toward increased magnetization transfer with increased concentration (1.1 to 1.25 - p=0.65). Finally, ADC analysis of the collagenase treated group revealed an increase with increasing concentration (from 1.4 10^-11m^2/s to 1.65 10^-11m^2/s). Trypsin degradation resulted in a more subtle increase in the ADC results noted (from 1.4 10^-11m^2/s to 1.6 10^-11m^2/s).

CONCLUSION: These results are the first reproduction of quantitative MR changes noted with human disc degeneration using directed controlled enzymatic techniques. The effects noted are enzyme and concentration specific. Given the constant percentage of water content throughout all discs, the change in quantitative MR signal is a result of the change in matrix integrity induced by these enzymes. The drop in T1 and T2 relaxation times imply that spin/spin and spin/lattice proton relaxation is rendered significantly shorter with the degradation of the matrix. The fact that collagenase induces an effect on magnetization transfer supports the theory that the signal is at least partly due to the hydroxyl / water interaction on the collagen chains. With the alteration of the collagen network, the transfer of protons from the bound to the free pool increases. ADC was also increased slightly with both collagenase and trypsin, indicating a facilitation of the proton diffusion through the matrix. Although preliminary, these findings further our understanding of the effect matrix components and matrix integrity have on the quantitative MR signal being generated. This represents a significant advancement in our attempt to further MR’s role as a powerful diagnostic tool of disc degeneration.


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