INTRODUCTION

Diseases of the spine that result from disc degeneration afflict a large proportion of the adult population. The development of magnetic resonance imaging (MRI) as an accurate and non-invasive diagnostic tool of the disc matrix composition and structural integrity will allow the detection of early disc degeneration as well as the evaluation of new treatment strategies. T1rho is a recently established MR protocol that measures the spin-lattice relaxation time in the presence of a spin-lock radio-frequency field, and has been correlated with proteoglycan loss in degenerated discs [1]. The nucleus pulposus (NP) of the disc, which is rich in proteoglycan, shows a distinct decrease in proteoglycan content with disc degeneration [2]. The purpose of this study was to assess the biochemical and mechanical properties of trypsin-treated discs under physiological load using T1rho.

METHODS

Experimental Groups:

Bovine caudal segments (n=6; 2-3 years-old) as 3-disc segments were injected in the NP with either 5 mg of trypsin (Sigma, ON, Canada) in 40 µl Tris buffer or with Tris buffer only. The 3-disc segments were placed in bags containing saline solution and antibiotics and were kept at 37°C throughout the experiment. The segments were subjected to either 16h of cyclic compression loading (50N–300N–50N at 1Hz; n=3) or were left unloaded (n=3) for 16h. The segments were then paraffin embedded for MRI.

MRI Procedure:
The MR examinations were carried out in a 1.5T whole-body Siemens’ Avanto system using the standard circularly polarized head coil. For T1rho, a series of T1rho-weighted images were acquired using a non-compensating turbo-spin-echo sequence [1] with TE/TR of 3000 ms/12 ms, six TSL of 10-80 ms, and B0 set at 500 Hz.

Biochemical Composition:
The discs were dissected and the NP and annulus fibrosus (AF) were separated for biochemical and mechanical analyses. The tissues were analyzed for contents of water, glycosaminoglycan (GAG), total collagen, and denatured collagen [2,3].

Mechanical Testing Procedure:
The portions of NP and AF tissues for mechanical analysis were immediately frozen on dry ice and kept at −80°C until analysis. Each NP and AF tissue plug of 5-mm diameter and 2-mm thickness was prepared using a cryostat and biopsy punch, and tested under confined compression as previously described [3]. Swelling pressure, compressive modulus Hs, and hydraulic permeability k were obtained using a ramp stress-relaxation experiment.

Statistical Analysis:
ANOVAs were used to evaluate the effects of loading condition and enzyme treatment on T1rho (P ≤ 0.05). Correlations were investigated between MRI parameters, biochemical properties and mechanical variables using Pearson tests (P ≤ 0.05).

RESULTS

Mechanical loading significantly decreased T1rho in both NP and AF tissues (P < 0.0001) (Fig. 1). Trypsin treatment of the NP had no effect on the T1rho parameter of NP and the adjacent AF, when compared to the buffer treatment (Fig. 1).

When all NP data were combined, a significant correlation was established between water and T1rho (r = 0.595, P = 0.01), and this correlation was also observed in trypsin-treated NP (r = 0.744, P = 0.02). In loaded and unloaded trypsin-treated NP, k correlated with T1rho (r = 0.514, P = 0.005). The compressive modulus of loaded NP, but not unloaded NP, correlated with T1rho (r = 0.776, P = 0.011).

When all AF data were combined, a significant correlation was detected between water and T1rho (r = 0.588, P = 0.012). The hydraulic permeability of loaded AF, but not unloaded AF, inversely correlated with T1rho (r = -0.768, P = 0.013).

DISCUSSION

The present study demonstrates for the first time a strong sensitivity of T1rho to compositional and structural changes in the disc associated with 16 hours of mechanical loading and a small effect associated with compositional and structural changes in the disc associated with trypsin treatment. T1rho reportedly correlates strongly with disc proteoglycan content, age, and grade of degeneration and moderately with water content [1]. In cartilage, T1rho inversely correlates with proteoglycan content [4]. In the present study, the only correlation of GAG with T1rho was found with AF that was adjacent to buffer-treated NP, whether loaded or not.

Digestion of NP by trypsin, which cleaves the proteoglycan core protein and does not attack intact collagen, decreased slightly GAG and water contents in loaded NP only (P < 0.05). This suggests that trypsin-degraded proteoglycan fragments may have diffused out of the disc only when loaded and are consistent with our previous findings that NP proteoglycan and water contents were reduced in mechanically loaded specimens [3]. While trypsin digestion of the NP did not affect T1rho values when compared to that of buffer-treated NP, T1rho correlated with water content and hydraulic permeability of trypsin-treated NP.

The compressive modulus of loaded NP correlated with T1rho, while the hydraulic permeability of loaded AF inversely correlated with T1rho. In cartilage tissue, proteoglycan content, compressive modulus and permeability all inversely correlated with T1rho [5]. Water and permeability are directly related and a previous study demonstrated T1rho was influenced by changes in hydration and in collagen orientation, particularly in complex matrices that undergo degradation [6]. Thus, in the present system under trypsin digestion, T1rho appears to be predominantly influenced by the NP water content.

This study demonstrates specific relationships of T1rho with structural and compositional changes in the disc and complements previous studies showing that loading also affects other MR parameters, i.e. T1, T2, MTR and diffusion [7]. Further studies are required to determine the potential of the T1rho technique to be used as a non-invasive diagnostic tool of the biochemical and mechanical changes occurring in disc degeneration.

REFERENCES


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