Recapitulating the Spectrum of Intervertebral Disc Degeneration in a Large Animal Model

Introduction

Intervertebral disc degeneration is a progressive cascade that leads to structural and mechanical failure, and is frequently associated with low back pain. Patients present in a variety of degenerative states, classically defined by the Pfirrmann grading system, based on qualitative interpretation of magnetic resonance images (MRI). Current treatments focused on alleviating the symptoms of discogenic pain are limited, as they do not restore disc mechanics or structure. As a result, there is considerable interest in developing biologic regenerative therapies to treat disc degeneration. For these therapies to be successful they must be tailored to the degenerative state of each disc. For example, they may include injection of stem cells, hydrogels or growth factors for mild to moderately degenerated discs, or engineered total disc replacements for severely degenerated discs. A critical aspect of evaluating and translating these regenerative approaches into clinical use is a size-relevant large animal model that recapitulates the spectrum of degeneration seen in humans. The objective of this study was to establish a goat model of intervertebral disc degeneration in which a gradient of degenerative changes, from mild to severe, could be reproducibly achieved through mechanical (nucleotomy) or chemical (chemonucleolysis) perturbation.

Materials & Methods

With IACUC approval, 9 goats underwent a surgical procedure to induce the degeneration of the lumbar intervertebral discs. Using an open, lateral, retroperitoneal transposaot approach, L1-2, L2-3 and L3-4 lumbar discs were randomized to receive either subtotal nucleotomy (n = 10) or injection of 200μL of either 0.1U (n = 5), 1U (n = 10) or 5U (n = 5) chondroitinase-ABC (ChABC) via a 22G spinal needle. The L4-L5 (n = 5) disc received a sham saline injection, and the T13-L1 and L5-L6 discs served as intact controls (n = 10). Lateral plain radiographs of the lumbar spine were obtained post-operatively, immediately post-operatively, and at 1, 2, 4, 6, 8, 10 and 12 weeks post-operatively for quantification of disc height index (DHI). After 12 weeks, the animals were euthanized, and the lumbar spines harvested and imaged using a 3T MRI scanner. Images for quantitative T1 and T2 mapping were obtained, as well as T2 weighted images for Pfirrmann grading. Correlations between quantitative MRI parameters and Pfirrmann grade were established by linear regression.

Results

Histological evaluation (Figure 1) revealed advanced degenerative changes to the disc in the 5U ChABC group, with moderate changes in the nucleotomy and 1U groups, and mild changes to the 0.1U ChABC group. Histologic scores were highest in the 5U and 1U ChABC group, followed by the nucleotomy and 0.1U ChABC groups across all categories. There were no statistically significant differences in histologic score between control and sham discs. Discs injected with 1U or 5U of ChABC exhibited a progressive decrease in DHI, with 28% and 34% reductions in DHI in the 1U and 5U groups, respectively, after 12 weeks (p < 0.05). Compared to the sham group, DHI was also significantly reduced in the 5U group at 10 weeks, and in the 1U group at 2, 4, 6, 8, and 10 weeks (p < 0.05). Discs subjected...
Figure 1. Histology. Degeneration was characterized by fibrosis of the nucleus pulposus, disorganization of the annulus fibrosus, loss of disc height and reduction in alcian blue staining. No degenerative changes were observed in sham discs compared to control. Alcian blue and picrosirius red stain, scale bar = 3 mm.

Figure 2. Microcomputed tomography (µCT). 3D reconstructions (A) of the subchondral cortical endplate and the adjacent trabecular bone illustrate alterations to the cortical (B) and trabecular (C) bone volume fraction. The cortical endplate with the lowest BV/TV is shown in (A). Bars denote significance, $p < 0.05$, ANOVA, Tukey’s post-hoc test.
to nucleotomy or injection of 0.1U ChABC also exhibited an initial decrease in DHI (significant at 1, 4, 6, 8 and 10 weeks); however, partial recovery of disc height was observed at 12 weeks in both groups. DHI for control and sham injected discs was unchanged over 12 weeks.

Three-dimensional μCT analysis (Figure 2) demonstrated significant cortical bone loss in the vertebral endplate of the 1U and 5U groups. The bone volume fraction of the adjacent trabecular bone was significantly increased for these same groups. T1 and T2 values in the nucleus pulposus (NP) were significantly lower in the 1U and 5U groups compared to sham and control discs. Nucleus pulposus T2 values were significantly lower in the nucleotomy group compared to sham. T1 and T2 values correlated significantly ($r^2 = 0.63$ and $r^2 = 0.53$, respectively) with Pfirrmann grades. Average force displacement curves (Figure 3) illustrated alterations to the mechanical response of the disc to loading in the 1U ChABC and nucleotomy groups compared to control. Compressive and neutral zone modulus were significantly lower, and range of motion greater, in nucleotomy and 1U ChABC discs compared to controls.

Discussion

A large animal goat model of disc degeneration was established that exhibits a gradient of degenerative changes from mild to severe. This work advances previous caprine animal models of degeneration, which have mainly achieved only mild degenerative changes.7 Observed changes to both the disc and the bone in the adjacent vertebral endplate are consistent with the changes reported in human disc degeneration.8,9 Over the 12 week study, nucleotomy and injection of low dose (0.1U) ChABC induced mild to moderate degenerative changes to the disc, as seen via disc height changes, quantitative MRI, and histology. Injection of higher doses (1U and 5U) ChABC induced moderate to severe disc degeneration. Structural derangement of the disc with degeneration in the 1U ChABC and nucleotomy groups was associated with significantly altered disc mechanical function.

Conclusions

In this study, we established a large animal model that replicates the spectrum of disc degeneration seen in humans and provides the basis for future studies of the biological mechanisms underlying disease progression. Moreover, this model can be used to evaluate the therapeutic potential and safety of a wide range of novel biological therapies designed to treat disc degeneration at a variety of stages.

References