

Percutaneous Delivery of Chondroitinase ABC Induces Moderate Disc Degeneration in a Goat Model

¹Andrew H. Milby, MD
^{1,2}Brent L. Showalter, BS
¹Joanne Haughan, VMD
^{1,3}Robert L. Mauck, PhD
³Dawn M. Elliott, PhD
¹Neil R. Malhotra, MD
¹Thomas P. Schaer, VMD
^{1,3}Lachlan J. Smith, PhD

¹University of Pennsylvania,
Philadelphia, PA, USA

²University of Delaware, Newark, DE, USA

³Philadelphia VA Medical Center,
Philadelphia, PA, USA

Introduction

Low back pain is highly prevalent, substantially impairs quality of life and is a leading cause of healthcare expenditures.¹ Low back pain leads to 15 million physician visits annually with only 500,000 patients meeting criteria for surgical intervention.² Four million patients will not meet surgical criteria, have moderate disc degeneration, and remain unresponsive to long term conservative treatment. Without an effective treatment paradigm for this large population, the community of spinal therapeutic specialists is focused on the development of minimally invasive strategies to treat and/or reverse moderate disc degeneration. Many approaches focus on injectable therapeutics for the nucleus pulposus (NP) that can normalize disc mechanical function, attenuate chronic inflammation, and/or potentiate native tissue regeneration.^{3,4} For in vivo evaluation of such therapeutics, a preclinical large animal that recapitulates key characteristics of moderate human disc degeneration is required. The large frame goat represents an attractive model given its disc geometry that is comparable to that of the human and that it provides a clinically relevant model for percutaneous approaches. Catabolic mediators such as chondroitinase ABC (ChABC) and interleukin-1 beta (IL-1 β) have shown promise in vitro and in vivo in instigating moderate disc degeneration, including loss of disc height and glycosaminoglycan content, and altered biomechanical properties.⁵⁻¹⁰ The objectives of this study were to evaluate a minimally-invasive, percutaneous technique for the introduction of catabolic agents into the goat NP, and to compare the efficacy of ChABC and IL-1 β as initiators of moderate disc degeneration.

Methods

Animal studies were performed following IACUC approval. Large frame goats (n=11) were placed in right lateral recumbency under general anesthesia. Lateral fluoroscopic images were used to verify number of lumbar vertebrae and levels for injection using the ribs and the sacrum as anatomical landmarks. Following a 5mm skin incision, an 18 gauge spinal needle with a blunt trocar was advanced via a posterolateral

approach under fluoroscopic guidance and docked on the outer annulus fibrosus. The trocar was removed, and a 22 gauge spinal needle was introduced into the center of the NP (Figure 1) under fluoroscopic guidance and distinct tactile feedback. Treatment groups were: ChABC, 1 U/ml in saline (L1-2 or L3-4, randomized); 2) saline sham (L2-3); 3) IL-1 β , 100 ng/ml in saline (L1-2 or L3-4, randomized); and 4) intact (non-injected) control (L4-5). The injection volume was 100 μ l. Animals were returned to normal housing and euthanized 12-weeks post-surgery. Lateral radiographs of the lumbar spine were obtained pre-operatively, and at 6 and 12-weeks post-operatively. Changes in disc height index (DHI) at the 6 and 12 week time points relative to pre-operative values were calculated using a custom Matlab program. Measurements were performed independently by 2 blinded assessors. Following euthanasia, 7 spines were imaged using magnetic resonance imaging (MRI). Series of T1rho and T2-weighted images were acquired on a 3T MR Scanner (Trio; Siemens) using turbo spin-echo sequences. The T2 images used TR=3000 ms and 6 evenly distributed echo-times between 13 and 78 ms, while the T1rho images used TR/TE=3000/12 ms with 5 evenly distributed spin-lock pulse durations from 12-60ms with a 500 Hz spin-lock pulse amplitude. T1rho and T2 scores were obtained using a regression of intensity data within the NP from the images. The remaining 4 spines were processed for paraffin histology. Mid-sagittal sections from

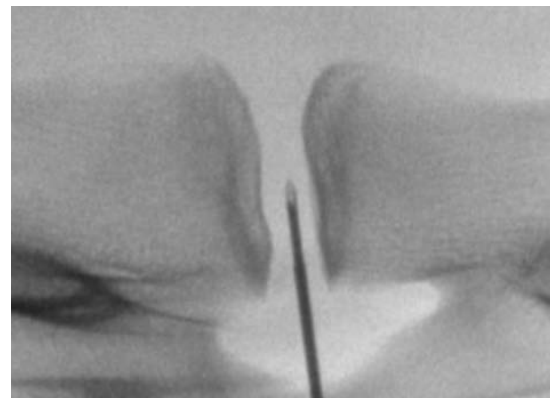


Figure 1. Placement of 22 gauge needle in the NP under fluoroscopic guidance.

each disc were stained with alcian blue (GAG) and picosirius red (collagen), and qualitatively assessed for morphological changes consistent with degeneration. Differences in DHI changes after 6 and 12 weeks, and differences in T1rho and T2 scores after 12 weeks between treatment groups were established using repeated measures ANOVAs with post-hoc, pairwise Student Neumann Keul's tests.

Results

For quantitative MRI assessments, T1rho scores were significantly different between groups ($p=0.009$), with post-hoc tests revealing that ChABC was significantly lower than all other treatment groups and the intact group (Figure 2A). T2 scores (Figure 2B) showed a trend toward decreases with ChABC treatment, but did not reach the level of significance compared to intact ($p = 0.06$). One spine was excluded from MRI analysis due to signal artifact. No significant changes in radiographic DHI were found between groups at either 6 or 12 weeks post-operatively. A high degree of inter-assessor variability was observed for DHI measurements, likely due to the inconsistent orientations of discs within the radiographic field of view, and the small effect size. With respect to histological evaluations, no signs of degeneration were apparent for any group (Figure 3).

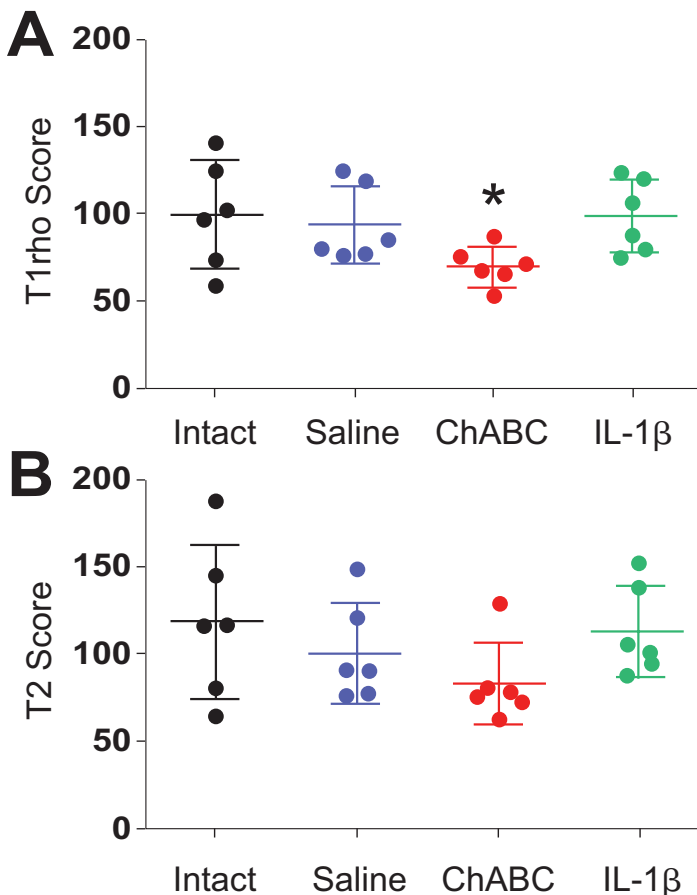


Figure 2. Quantitative MRI assessment of disc condition. A) T1rho scores. B) T2 scores. * $p<0.05$ vs all other groups.

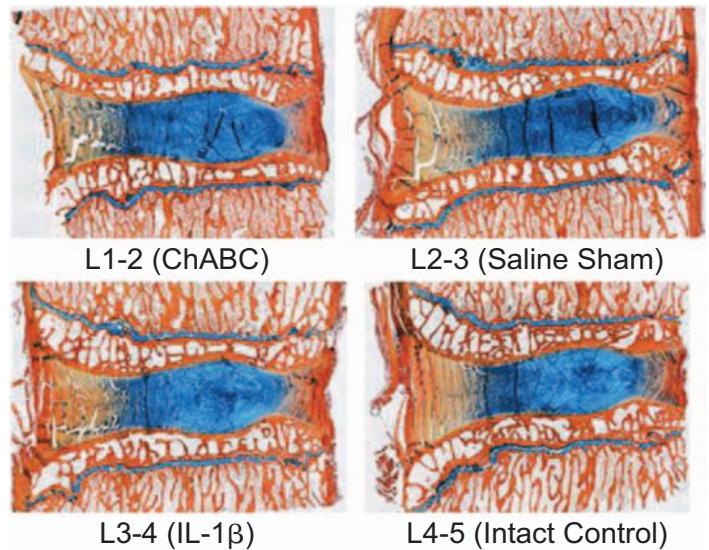


Figure 3. Histological assessment of disc condition. No morphological differences were apparent between treatment groups.

Discussion

In this study, we used a percutaneous surgical approach to initiate moderate degeneration in the goat disc. This minimally invasive, clinically translatable approach allows for the successful delivery of injectable agents to the disc space with an acceptable safety profile. Ongoing work will seek to further refine this model, and ensure accurate and repeatable delivery of agents to the goat NP. Previously, our team has successfully used both ChABC and IL-1 β to induce glycosaminoglycan loss in vivo in the rat disc.^{9,10} The current findings in the goat suggest that IL-1 β is less effective at inducing disc degeneration than ChABC, at least at the dosage evaluated in this pilot study. Despite this limitation, our results show that quantitative MRI is a sensitive method for identifying early degenerative changes in this model compared to both histological assessment and measurements of disc height from radiographs. Higher dosages of ChABC may be required to induce more consistent, measurable degenerative changes. Following further evaluation of this model (including biomechanical and biochemical assays), future studies will refine the model and use this platform to evaluate novel, minimally invasive therapeutics for disc degeneration, including bioactive hydrogel implants that are currently under development by our group.

Significance

Low back pain resulting from intervertebral disc degeneration is a significant socio-economic burden. The large animal model of moderate disc degeneration developed herein provides an important preclinical platform in which to evaluate minimally-invasive therapeutics for disc regeneration.

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