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A large animal model that recapitulates the spectrum of human intervertebral disc degeneration

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SUMMARY

Objective: The objective of this study was to establish a large animal model that recapitulates the spectrum of intervertebral disc degeneration that occurs in humans and which is suitable for pre-clinical evaluation of a wide range of experimental therapeutics.
Design: Degeneration was induced in the lumbar intervertebral discs of large frame goats by either intradiscal injection of chondroitinase ABC (ChABC) over a range of dosages (0.1U, 1U or 5U) or subtotal nucleotomy. Radiographs were used to assess disc height changes over 12 weeks. Degenerative changes to the discs and endplates were assessed via magnetic resonance imaging (MRI), semi-quantitative histological grading, microcomputed tomography (μCT), and measurement of disc biomechanical properties.
Results: Degenerative changes were observed for all interventions that ranged from mild (0.1U ChABC) to moderate (1U ChABC and nucleotomy) to severe (5U ChABC). All groups showed progressive reductions in disc height over 12 weeks. Histological scores were significantly increased in the 1U and 5U ChABC groups. Reductions in T2 and T1ρ, and increased Pfirrmann grade were observed on MRI. Resorption and remodeling of the cortical boney endplate adjacent to ChABC-injected discs also occurred. Spine segment range of motion (ROM) was greater and compressive modulus was lower in 1U ChABC and nucleotomy discs compared to intact.
Conclusions: A large animal model of disc degeneration was established that recapitulates the spectrum of structural, compositional and biomechanical features of human disc degeneration. This model may serve as a robust platform for evaluating the efficacy of therapeutics targeted towards varying degrees of disc degeneration.

Introduction

Degeneration of the intervertebral discs is implicated as a leading cause of low back pain, a prevalent condition that places a significant social and economic burden on the individual and society at large[1,2]. Each year in the United States, 15 million patients present to a physician with a complaint of low back pain. Of these patients, only 500,000 have late-stage degeneration and meet the criteria for surgery[3]. Surgical interventions, most commonly fusion of the affected motion segment or artificial total disc replacement, are limited in that they do not restore native disc structure or function, and treatment may exacerbate degeneration of adjacent healthy discs[4]. Additionally, nearly 4 million patients who are not candidates for surgery have varying degrees of mild to moderate disc degeneration that is not

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responsive to conservative treatment. There is therefore a substantial unmet clinical need for new treatment options for disc degeneration and low back pain.

Disc degeneration is a progressive cascade of cellular, biochemical, structural, and biomechanical changes that manifests as a spectrum from mild to severe. It is thought that the earliest degenerative changes typically occur in the central nucleus pulposus (NP), and include loss of proteoglycan and water content, which compromises the ability of this disc compartment to swell and effectively engage the surrounding annulus fibrosus (AF)[5,6].

Because of the lack of therapeutics targeting mild- to moderate-stage degeneration, and the shortcomings of surgical solutions for late-stage degeneration, there exists considerable research interest in developing new, biological regenerative treatment strategies. These treatment strategies must aim to provide both pain relief and long-term regeneration of disc structure and mechanical function, and may include, for example, stem cell or growth factor injections into the disc, hydrogels for NP replacement or augmentation, and tissue-engineered total disc replacements[7–9].

For these therapies to be successful, they must be tailored to the degenerative state of the disc in need of treatment. As such, preclinical animal models, an essential step for translating emerging therapies to humans, should effectively recapitulate the spectrum of degeneration, including reproducible structural, compositional and biomechanical changes[10]. Animal models of disc degeneration range in scale from small species, such as the mouse, rat, and rabbit, to large species, including the sheep, goat, pig, and dog[10–14]. The benefits of large animal models include similarities in morphology to the human lumbar disc as well as sufficient disc height to mimic the challenging nutritional environment present in human discs[15]. Furthermore, in the sheep and goat, NP proteoglycan content and whole-disc mechanics are similar to human[16,17]. Certain goat breeds, specifically, are an attractive model due to greater disc height when compared to similar sized species such as pigs and sheep, and the absence adult notochordal cells[18].

A variety of approaches to induce disc degeneration have been utilized in both large and small animal models, including chemonucleolysis (to enzymatically induce NP degradation), nuclear aspiration, annular injury, or altered mechanical loading. Annular injury models (laceration or needle puncture) have been characterized in mice, rats, rabbits, sheep and goats, with the extent of degeneration dependent on the size of the injury relative to the size of the disc[13,19–21]. Chemonucleolysis, most commonly achieved via intradiscal delivery of chondroitinase ABC (ChABC) or chymopapain, has been utilized in an effort to recapitulate the hallmark loss of proteoglycans that occurs in human disc degeneration[11,22]. ChABC specifically degrades the chondroitin and dermatan sulfate side chains of proteoglycans, and has yielded mild to moderate degeneration in rats, rabbits, sheep, goats, and dogs, although spontaneous regeneration has been observed in smaller species[18,23–27]. Chymopapain is a proteolytic enzyme that cleaves the non-collagenous protein connections of proteoglycans, and induces degeneration in dogs that is typically more severe than that achieved via ChABC[22,28]. A spectrum of degeneration has also been achieved via chymopapain in *in vitro* organ culture models[29]. Previous models of intradiscal ChABC injection in large animals, such as the goat, sheep, and dog, are primarily characterized by mild degenerative changes[18,24,25]. Therefore, a spectrum of degeneration, which has utility for the evaluation of a variety of pre-clinical regenerative therapeutics, has yet to be achieved in a large animal model via these methods. Thus, the purpose of this study was to establish an inducible large animal model that reproducibly recapitulates the spectrum of intervertebral disc degeneration found in human patients.

Methods

Animals and surgical procedures

The study was approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. Nine large frame castrated male goats (Thomas D. Morris Inc., Reisterstown, MD), approximately 3 years-of-age, were utilized in this study. Except for surgical procedures and radiographs, animals were group housed in a barn, and evaluated daily by a veterinarian for signs of pain, behavior changes or gait abnormalities for the duration of the study. All animals underwent a surgical procedure to induce degeneration of the lumbar intervertebral discs, as per the study design in Fig. 1(A). Animals were anesthetized via intravenous injection of ketamine (11–33 mg/kg) and midazolam (0.5–1.5 mg/kg), and then intubated and maintained on an isoflurane–oxygen mixture throughout the surgical procedure. Using standard aseptic technique, the lumbar intervertebral discs were exposed via an open, left lateral retroperitoneal, transpsoatic approach. The disc spaces were identified and counted using lateral fluoroscopy, and a titanium Kirschner wire was placed in the L1 or L2 vertebral body as a fiducial marker to enable identification of vertebral levels on radiographs [Fig. 1(B) and (C)]. As indicated in Fig. 1(A), the L1–L2, L2–L3 and L3–L4 and L4–L5 discs were randomized to receive either subtotal nucleotomy (i.e., a nuclectomy) ($n = 10$), sham injection ($n = 5$), or injection of 0.1U ($n = 5$), 1U ($n = 10$) or 5U ($n = 5$) of ChABC (Amsbio, Cambridge, MA). The T12–L1 and L5–L6 discs served as intact controls ($n = 10$), for a total of six experimental groups. Each dose of ChABC was resuspended in 200 μL of vehicle (sterile PBS containing 0.1% BSA) for injection, and delivered to the NP using a 6-inch, 22G spinal needle. Sham injection consisted of 200 μL of vehicle only. Subtotal nucleotomy was performed using a cruciate annular incision followed by 2 mm pituitary rongeurs to remove NP (0.43 ± 0.17 g). The surgical incision was then closed in layers, and the animals were hand-recovered by veterinary staff until ambulatory, upon which they were returned to standard housing. Peri-operatively, animals were administered transdermal fentanyl (2.5 mcg/kg/hr) and intravenous flunixin meglumine (Banamine, 1.1 mg/kg) for analgesia. Florfenicol (40 mg/kg) was administered for antimicrobial prophylaxis.

Radiographs and magnetic resonance imaging (MRI)

To assess longitudinal changes in disc height with degeneration, lateral plain radiographs of the lumbar spine were obtained in the standing and fully weight-bearing position pre-operatively, post-operatively and at 1, 2, 4, 6, 8, 10, and 12 weeks post-operatively. Disc height index (DHI) was quantified by a blinded assessor using a custom MATLAB program (Mathworks, Natick, USA)[30]. Twelve weeks post-operatively, animals were euthanized by an overdose of pentobarbital solution according to American Veterinary Medical Association guidelines[31], and the lumbar spines harvested. The lumbar spines were imaged using a 3T clinical MRI scanner (Siemens Magnetom TrioTim, Munich, Germany) with a voxel size of 0.6 mm × 0.6 mm × 5 mm. T2-weighted mid-sagittal images were obtained for Pfirrmann grading. Series for T2 (Echo Time = 13 * i, i = 1, 2 … 6) and T1ρ (Spin Lock Time = 12 * i, i = 1, 2 … 5) mapping were also obtained, and the T2 and T1ρ relaxation times were quantified in a manually segmented circular region of interest in the NP using ImageJ (NIH, Bethesda, USA) as previously described[32]. Following MRI, each lumbar spine was divided into vertebra-disc-vertebra motion segments, vacuum sealed, and stored frozen at −20°C. Five motion segments from each experimental group were utilized serially for microcomputed tomography (μCT) and histologic analyses. Additional segments