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Basic Science

Disc degeneration reduces the delamination strength of the annulus fibrosus in the rabbit annular disc puncture model

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Abstract

BACKGROUND CONTEXT: Degenerative disc disease is a common pathologic disorder accompanied by both structural and biochemical changes. Changes in stress distribution across the disc can lead to annulus fibrosus (AF) damage that can affect the strength and integrity of the disc. Given that some present degeneration therapies incorporate biological regrowth of the nucleus pulposus (NP), it is crucial that the AF remains capable of containing this newly grown material.

PURPOSE: To examine the resistance of AF to delamination using an adhesive peel test in experimentally degenerated rabbit discs.

STUDY DESIGN: Experimentally induced disc degeneration; excised AF tissue study.

METHODS: Disc degeneration was induced in eight New Zealand white rabbits by annular puncture; four additional rabbits served as controls. In experimental rabbits, an 18-gauge needle was inserted into the anterolateral AF region of levels L2–L3 and L4–L5, and disc height was monitored by X-ray. Animals were sacrificed at 4 and 12 weeks postsurgery and magnetic resonance images and X-rays were taken. Four discs were excised from the experimental animals; two punctured (L2–L3 and L4–L5) and two controls (L3–L4 and L6–L7). The same four discs were also excised from the age-matched control animals and served as nonpunctured control discs. To determine resistance to delamination, AF samples were dissected from each disc and subjected to a mechanical peel test at 0.5 mm/s.

RESULTS: Magnetic resonance imaging and X-ray images confirmed dehydration of the NP and reduced disc height, similar to that found in clinical degeneration. Resistance to delamination was significantly lower in punctured/degenerated discs compared with both the nonpunctured discs from the same animal (27% lower) and the nonpunctured control discs (30% lower) ($p=.024$).

CONCLUSIONS: The findings of this study suggest that degeneration increases the potential for delamination between AF layers. Given this substantial change to the integrity of the AF after degeneration, clinical treatments should not only target rehydration or regrowth of the NP, but should also target repair and strengthening of the AF to confine the NP.

Keywords: Annulus fibrosus; Lamellae; Disc herniation; Animal model; Puncture; Disc therapy

Introduction

Degenerative disc disease is a common pathologic disorder accompanied by both structural and biochemical changes that may be associated with aging, biomechanical insult, and genetic background [1]. Disc degeneration is biologically mediated with complex biochemical and inflammatory processes that alter the homeostasis of the disc's extracellular matrix [2,3]. Progression of degeneration subsequently

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affects the composition of the disc. Reduced proteoglycan content in the disc [4] and thus, decreased hydration and disc height loss, increased collagen Type I in the inner half of the annulus fibrosus (AF) and nucleus pulposus (NP), and increased collagen cross-linking [5] are all associated with degeneration progression. Mechanical factors (ie, vibration, torsion, and compression/loading) are also implicated in the induction of disc degeneration [6,7].

Mechanical adaptations subsequently occur because of these biochemical changes in the disc. Specifically, as hydration levels in the NP drop, loading patterns on the disc are altered, resulting in abnormal shear and compressive stresses experienced by the AF rather than the NP [8–10]. These changes in stress distribution across the disc are likely the main culprit of structural damage, including increased delamination [11], fissure formation [12], and inward lamellae buckling [13], which have been observed in the AF after degeneration. Delamination, specifically, is characterized by the separation of individual layers in the AF from each other as a result of failure of the adhesive bond. If this occurs in the disc, the integrity or strength of AF is likely compromised, thereby increasing the risk of further damage.

The focus of the present study was to examine the effect of disc degeneration on the resistance to delamination in the AF. The examination of structural changes to the disc, and in particular the AF, as a result of degeneration is of considerable importance because most structural damage is irreversible in the aging disc. This is because of significantly slowed metabolism and extracellular protein synthesis [4,14], preventing sufficient healing despite evidence of increased cytokine and matrix metalloproteinase secretion [15]. By understanding the effects of degeneration on the disc, biological repair therapies can be developed to allow for more specific target of the weakened structures. Therefore, the purpose of this study was to determine the peel strength of the interlamellar matrix, and thus resistance to delamination, in rabbit AF tissue after induced degeneration. A unique peel test design [16] was used to measure the delamination strength of the interlamellar matrix of the AF after degeneration development. In short, this test allows for the quantification of the material properties of the matrix found between layers of AF by employing a 180° mechanical peel test.

Methods

Animal model

An established annular puncture intervertebral disc degeneration model in the New Zealand white rabbit (Western Oregon Rabbit Co., Philomath, Oregon, USA) was used for the present study [17]. Specifically, eight rabbits (4.6±0.2 Kg) underwent spinal surgery to induce disc degeneration (termed experimental animals) and four additional rabbits (4.7±0.2 Kg) served as control animals. The study protocol was approved by the University Animal Care Use Committee.

Four of the experimental animals underwent spinal surgery at 8 months of age and were sacrificed 1 month postsurgery (9 months of age), whereas the remaining four experimental animals underwent surgery at 6 months of age and were sacrificed 3 months postsurgery (therefore also 9 months of age at the time of sacrifice). The four control animals were sacrificed at 9 months of age and did not undergo any surgical operation.

Annular puncture disc degeneration model

Before spinal surgery, animals were anesthetized with xylazine (5 mg/Kg; Akron, Inc., Decatur, IL, USA) and ketamine hydrochloride (35 mg/Kg; Fort Dodge Animal Health, Fort Dodge, Iowa, USA) by subcutaneous injection. Anesthesia levels were maintained during surgery with 1% to 3% isoflurane (Piramal Critical Care, Boise, ID, USA). Each rabbit was positioned laterally on its right side, and a sagittal plane radiograph was taken to locate the L3–L4 intervertebral disc. An incision (approximately 6 cm in length) was made along the posterolateral side of the animal (right side of body) into the retroperitoneal space. Using the L3–L4 disc as a guide, the L2–L3 and L4–L5 discs were located and subsequently punctured with an 18-gauge needle through the anterolateral region of the AF [17]; Fig. 1, Top Left. Care was taken to ensure the needle did not puncture the posterior AF tissue. After disc punctures, the surgical site was cleaned with sterile saline and closed using layered sutures. Each rabbit was then given a subcutaneous injection of buprenorphine hydrochloride (analgesic; 0.05 mg/Kg; Reckitt Benchkiser Pharmacueticals, Richmond, VA, USA) and Cefazolin (antibiotic; 22 mg/Kg; Steri-Pharma, LLC, Syracuse, NY, USA) and monitored every 15 minutes (heart rate, blood oxygen, body temperature, and respiratory rate). Once fully alert, the animals were returned to their cages and allowed to resume regular daily activity. Additional doses of buprenorphine hydrochloride and Cefazolin were given 1 and 2 days postsurgery. Animals were monitored each day until sacrifice.

Image analysis

Radiographs and disc height

Disc height was monitored by lateral radiographs taken 1, 2, 4 (for all experimental animals), 8, and 12 weeks postoperative (only for the four animals that were sacrificed 3 months postsurgery) under anesthesia with ketamine hydrochloride (35 mg/Kg) and acepromazine maleate (1 mg/Kg; VEDCO, Inc., St. Joseph, MO, USA). Care was taken to ensure similar level of anesthesia for each radiograph. All animals were sacrificed while anesthetized via venous (marginal ear vein) injection of Euthasol (1 mL/4.54 Kg; Virbac Animal Health, Fort Worth, TX, USA). Disc height loss was determined by calculating the disc height index (DHI) [17] for each radiograph and comparing it with the DHI just before surgery.