Introduction

- Models of acute annulus fibrosus (AF) injury consistently produce nuclear depressurization and biological remodeling similar to that of human disc degeneration. These methods are easily implemented, provide repeatable results and have controlled specificity in comparison to global knockout and spontaneous development models.
- AF injury studies have been conducted in many species including the sheep, rabbit, and rat1-4. In addition, the mouse caudal disc has been proposed as a model, as it degenerates as a result of needle puncture injury, displaying changes consistent with those in humans5.
- The primary function of the disc is mechanical - transferring load, dissipating energy and facilitating joint mobility. Thus evaluating disc mechanics is important to understanding how functional changes can be targeted by potential therapeutics. Limited mechanical data for puncture-initiated degeneration is available for the mouse, however.

OBJECTIVE

To quantify the acute and long-term effects of needle puncture injury on mouse caudal disc mechanical function, height, and nucleus pulposus (NP) glycosaminoglycan (GAG) content.

Materials and Methods

- Surgery - Retired breeder mice were allocated to two post-surgical time points: zero weeks (n=10) and eight weeks (n=5). The (caudal) C6/C7 and C8/C9 discs were either exposed and punctured with a 29G needle (+65% disc height) or exposed as an intact sham control. The needle was clamped and inserted 1.75 mm to ensure full penetration of the adjacent annulus.
- Mechanics - Spines were then separated into motion segments, equilibrated in PBS and 4°C overnight and tested in tension/compression (20 cycles: +0.5 N, -1.5N, 0.025 Hz), creep (-1.5 N, 1 hour), and torsion (10 cycles: +/-8°, 0.05 Hz) on an Instron 5542 mechanical testing system equipped with a custom torsional testing device. The device is composed of a stepper motor in series with a torque cell and two digital cameras to track the extension and rotation of each motion segment (Fig.1). Compression, creep, and torsion were analyzed for total ROM, creep displacement, and torsional stiffness respectively.
- Micro CT - Prior to testing, caudal spines were imaged en bloc with a vivaCT 40 preclinical microCT at an isotropic 21 μm resolution. Disc height (h) and polar moment of inertia (Iy) were measured using a custom Matlab program by converting 3D reconstructions across the disc space to image stacks (Fig.2). All mechanical parameters were normalized to disc geometry: total ROM and creep displacement were multiplied by 1/h while torsional displacement were multiplied by 1/h while torsional stiffness was multiplied by h/Iy.
- GAG Content - Each disc was dissected from the adjoining vertebral bodies using a cryostat microtome. A 0.75 mm diameter biopsy punch was used to isolate the nucleus pulposus which was digested in proteinase K. Sulfated GAG content was then measured using the DMMB assay.
- Histology - Paraffin embedded motion segments were sectioned sagittally and stained with Alcian Blue and Picrosiris Red for GAG and collagen respectively and viewed in bright field and cross-polarized light.

Results

- Mean disc height was 19% lower for injured discs (p<0.05) compared to intact controls after eight weeks (Fig. 3). Disc height for shams also decreased after 8 weeks, but not significantly.
- GAG content was lower for injured discs (19%) and shams (17%) after 8 weeks compared to week 0 intact controls, but not significantly for either (Fig. 3).
- Total ROM was 32% greater for injured discs after 8 weeks compared to day 0 intact controls (p<0.05). ROM for sham treatment was also significantly greater after 8 weeks. Creep displacement was 34% greater for injured discs after eight weeks (p<0.05) than day 0 intact controls (Fig. 3). There were no differences in torsional stiffness for intact/sham and injured discs at 0 or 8 weeks.
- There were no differences histologically between sham and punctured discs at 8 weeks (Fig. 4).
- In general, puncture defects resulted in deficiencies in compressive properties, both elastic and viscoelastic, but no changes in torsional properties.

Discussion

- In this study an acute AF injury was used to create moderate degenerative changes in the mouse caudal disc. Reduction in GAG content (nonsignificant), which has been extensively correlated to degenerative grade, was measured as well as a corresponding decrease in disc height.
- For the zero week group, the 29G needle did not produce differences between control and punctured discs. In both compression and torsion, mechanics remain intact unless a puncture defect larger than some critical size is created. Then, mechanical effects are primarily dependent on the depressurization of the NP and the maintenance of AF tension. Puncture defects larger than the critical size will.
- In the murine caudal disc, the critical defect size (nucleus diameter to disc height ratio) for creating a mechanical effect is very large (~75-80%)7,8, much larger than what has been reported in the lumbar disc (~40%)9. This may be due to lower residual tension in the caudal AF. A larger needle is required to create depressurization because for smaller defects at lower nuclear pressures the resilient AF may have the ability to self seal.
- It has been suggested that the presence of sufficient mechanical perturbation is required for degenerative changes to take place8. Consistent with this theory, this study demonstrates that puncture defects that cause minor changes in mechanical properties e viv o, lead to moderate degenerative changes in vivo. This suggests that the biological response following puncture injury is more detrimental to disc mechanics than the puncture itself.
- Because there were no differences between sham and treatment groups, an examination of later timepoints is required to deduce whether these changes are a result of needle puncture and not a transient inflammatory response. Future work will consist of evaluating larger needle sizes and later timepoints.

References


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