Lumbar Disc Degeneration Induced by Annular Injury In An Ovine Model. Radiological Analysis and Comparison With Biochemical Changes.

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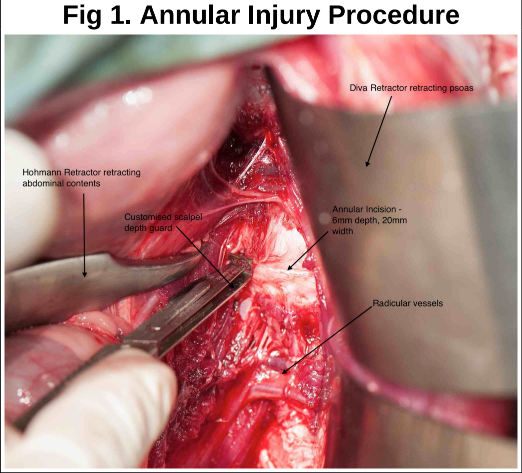
Introduction

Annular injury is commonly used to induce lumbar intervertebral disc degeneration in pre-clinical animal studies(1). We present a detailed radiological analysis of the changes, which occur in a three level ovine model of lumbar disc degeneration, and correlate this with biochemical changes.

Methods

6 sheep underwent standardised annular incisions (6mm by 20mm) at three adjacent lumbar levels (L2/3, L3/4, L4/5) via a left lateral retroperitoneal approach (Fig 1). L1/2 and L5/6 discs served as un-injured controls. X-rays were obtained prior to injury and 3 months post injury. 3T MR imaging was performed at 3 months following necropsy and analysed blindly using the Pfirrmann Disc Degeneration Scoring system(2). Three spines were examined histologically and the remaining spines dissected into the nucleus pulposus (NP) and annulus fibrosus (AF) for biochemical and histochemical analysis for proteoglycan (PG) content and extractability.

Fig 1. Annular Injury Procedure



Results

Injured discs (n=18) demonstrated a significant reduction in pooled Disc Height Index (DHI) of 0.02318 ($P<0.0001$), corresponding to a 23.67% mean decrease in DHI over the three-month period, whilst non-injured discs (n=12) demonstrated no significant change in DHI. In addition there was no significant difference in the change in DHI between injured levels (L2/3,L3/4,L4/5).

Median Pfirrmann MRI degeneration scores for the non-injured and injured groups were 1 and 2 respectively, and the difference between groups was significant ($P=0.0482$). In addition there was no differences between injured levels.

Fig 2. Disc Height Index

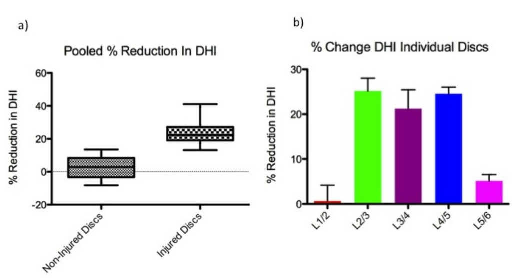


Fig 3. MRI Findings

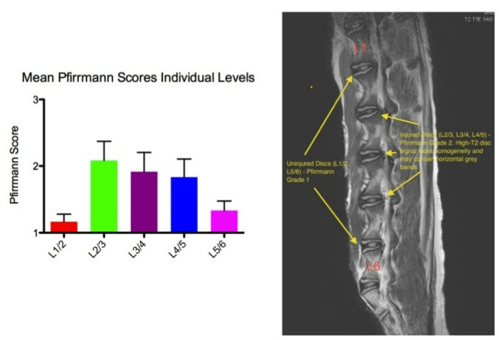
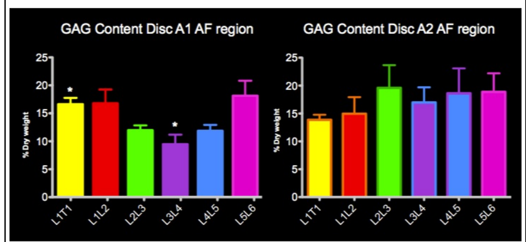


Fig 4. PGs in AF

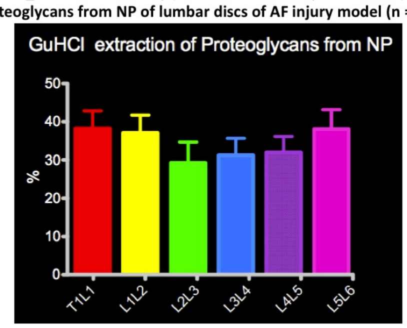
Mean ± SEM distribution of PGs in the AF1 and AF2 regions of the control and injured AF of discs of the sheep lumbar spines (n = 3)



Levels of PGs (measured as GAGs) in injured regions (A1) of L2L3, L3L4 & L4L5 discs lower than same AF region of Control discs L1T1, L1L2 & L5L6. Corresponding A2 AF Regions of the injured discs not affected (* p < 0.05).

Fig 5. PGs in NP

Mean ± SEM extractability (4M Guanidinium Hydrochloride) of Proteoglycans from NP of lumbar discs of AF injury model (n = 3)



Less Proteoglycans extracted from NPs of injured discs (L1L2, L3L4, L4L5) than controls

The proteoglycan (PG) content of the AF regions of injured discs was significantly lower than the corresponding region of the non-injured AF as well as the normal controls. In contrast the injured AF regions contained more DNA than the corresponding non-injured AF of the same discs or that of normal controls. The percentage of PGs extracted from the NP of injured discs was lower than that from the NP of control discs.

Discussion

The decrease in PG content of the NP and AF which occurs post annular injury is due to their proteolytic depletion which occurs in response to altered spinal mechanics at the injured level. Injured AF regions contain more DNA likely representing cell division and vascular invasion during the repair process. Similar biochemical processes occur in the early stages of disc degeneration in humans.

Imaging findings in this model also mimic early human disc degeneration where there is loss of disc height and a subtle loss of MRI T2 disc signal and homogeneity (Pfirrmann 2). In addition there is horizontal banding/fissuring evident within injured discs. In future studies we aim to include quantitative measures of disc degeneration on MRI analysis.

Importantly there were no significant biochemical or radioloigcal differences within the injured spinal levels (L2/3,L3/4,L4/5) or uninjured control levels (L1/2,L5/6).

Conclusions

MRI degeneration scores and changes in DHI are consistent with biochemical changes in PG content which occur 3 months post annular injury, in a three level ovine model of lumbar disc degeneration. We are using this model to study the efficacy of novel stem cell therapies to effect disc regeneration.

References

1. Melrose, J., et al., Recent advances in annular pathobiology provide insights into rim-lesion mediated intervertebral disc degeneration and potential new approaches to annular repair strategies. Eur Spine J, 2008. 17(9): p. 1131-48.3.

2. Pfirrmann, C.W., et al., Magnetic resonance classification of lumbar intervertebral disc degeneration. Spine (Phila Pa 1976), 2001. 26(17): p. 1873-8.