Detrimental Effects of Discectomy on Intervertebral Disc Biology can be Decelerated by Growth Factor Treatment during Surgery - A large animal organ culture model

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Disclosures:

Introduction: Lumbar intervertebral disc (IVD) herniation is a common spine disorder with a lifetime occurrence as high as 40%. The majority of IVD herniations improve over time or with non-operative therapy, yet a proportion of patients require surgical intervention [1]. While clinical studies have demonstrated benefits in relief of radicular symptoms post-surgery [1] little information is available on the effects of discectomy on the biology of the remaining IVD structure. The annular defect as well as the altered integrity of the remaining nucleus pulposus (NP) may promote or accelerate degenerative changes of the IVD resulting in additional long-term clinical problems [2] such as chronic low back pain. Development of cost effective strategies to prevent or reduce the severity of post-discectomy degeneration may dramatically improve outcomes and reduce healthcare costs. The objectives of this study were to improve knowledge of how discectomy procedures influence IVD physiology and to assess the potential of growth factor treatment as an augmentation at the time of surgery. Study one was a dose response study to assess the effects of TGFβ3 dose and sustained injection effects on an intact IVD using cell viability and gene expression. Study two assessed effects of discectomy with and without TGFβ3 augmentation using gene, cell viability, structural, and protein measurements.

Methods: Skeletally mature bovine caudal IVDs were obtained from a local abattoir and IVDs were dissected and prepared for organ culture. The screening study determined the optimal TGFβ3 dose. IVDs were randomly distributed among 6 groups (n=6) with 4 TGFβ3 doses (0.02, 0.2, 1 and 2 µg) in 100µl of PBS/0.1%BSA, and PBS-injected and un-injected controls. IVDs were placed in organ culture chambers [3] and cultured for 1 day (n=8) in standard high glucose DMEM, at 37°C, 5% CO2 and ambient O2. A 7 day study confirmed the chosen dose of 0.2 µg TGFβ3, with media changes every 3-4 days. Tissue was then taken for gene expression and cell viability assessments. The discectomy study determined the effect of discectomy and TGFβ3 on IVDs. IVDs were randomly distributed among 4 groups: d0, control, discectomy (injured), and discectomy + TGFβ3 (treated). For injured and treated IVDs, a discectomy was created on the dorsal side with a cruciate cut to the center of the IVD using a #15 blade, followed by tissue loosening and removal with a curette and rongeur (Fig.1). A total of 0.2 µg TGFβ3 in 20 µl PBS+BSA was injected into several locations of the discectomy site of treated IVDs. IVDs were loaded in bioreactors and cultured (as described above) for 1 (n=4), 6 (n=6) or 19 (n=9) days under diurnal loading (8h/16h = 0.1MPa/0.2MPa). To visualize the injection fluid distribution, 2 IVDs were injected with 20 µl FITC labeled dextran/PBS and cultured for 1 day. After culture annulus fibrosus (AF) and NP were separated. Only limited amounts of NP were available after discectomy and therefore excluded for further analyses. For cell viability analyses areas of treated and injured IVDs were divided in 2 categories: far field (treated/injured) and injury site (treated is/injured is) using procedures described previously [3,4]. Dextran distribution was visualized by fluorescence and second harmonic generation imaging of plastic embedded sections. Relative expression of selected anabolic and catabolic genes was quantified by real-time RT-PCR. Nitric oxide (NO) release into the media (Griess reaction), IVD height (before and after culture), aggrecan degradation, (Western blot) and proteoglycan content in tissue and media (DMMB) were determined. For all statistical analyses a Student’s t-tests with Bonferroni correction determined significance (p< 0.025).

Results: Screening study: TGFβ3 injection had a dose-dependent effect on matrix gene expression after 1 day culture, with increases in Aggrecan, MMP1 and ADAMTS4 at a dose of 0.2µg (p<0.05) and no differences observed for 0.02, 1 and 2 µg TGFβ3. The dose 0.2 µg TGFβ3 was considered optimal and at 7 days exhibited a general up-regulation for matrix genes with significance for Col1 and MMP1 (p<0.05; Fig. 2) and cell viability was maintained.

Discectomy study: Cell viability in control IVDs was maintained for up to 19 days. Discectomy led to significantly decreased AF viability both adjacent to the injury site (p<0.01) and to a lesser extent in the far field AF across from the injury (p<0.01). TGFβ3 treatment maintained cell viability during culture in the far field and mitigated cell death adjacent to the injury site (Fig.3). Fluorescence and SHG microscopy analyses demonstrated ruptures in the collagen rich matrix due to discectomy and revealed that dextran remained within areas close to the injection site. After 19 day culture, intact aggrecan was detected in all conditions, while degradation products were observed mainly in injured IVDs (Fig.4a). No difference in total NO release was
detected between control and treated IVDs (p=0.336). In contrast, untreated discectomy lead to a significant increase of total NO release (p<0.005). During the first week of culture, treated and injured IVDs released twice as much NO compared to control IVDs. With progressing culture time, NO release increased slightly in the control group, whereas it decreased in treated IVDs (Fig. 4b). IVD height decreased in all groups with height loss being highest in injured IVDs (22±4%) and lowest in treated IVDs (18±4%; p=0.06 injured vs. treated) groups. No differences in IVD height loss were observed between control (21±9%) and treated IVDs (p=0.79). The reduced height loss in treated IVDs is likely associated with inhibited MMP degradation of aggrecan (and possibly other proteins) following TGFβ3 injection.

**Discussion:** This study investigated the effects of discectomy on IVD biology and evaluated if TGFβ3 injection during discectomy could augment surgical procedures and reduce post discectomy degenerative processes. Post-discectomy degeneration is also supported by in vivo studies where experimentally induced annular puncture leads to significant changes in the biomechanical properties of IVDs. Our results indicated that discectomy procedures with NP tissue removal induced IVD height loss, GAG degradation, increase NO production and substantial cell death in injured IVDs, providing an injury model that simulates the clinical situation. TGFβ3 injection at the time of discectomy mitigated several of these degenerative changes with improved cell viability, ECM quality and reduced NO. Effects of discectomy should be interpreted with caution since discectomy on healthy bovine IVDs may produce greater degenerative changes than human IVDs which are typically somewhat degenerated at the time of discectomy. However, results identified that post-discectomy degeneration is likely to be associated with cell death and rapid matrix degradation. This study also demonstrated that TGFβ3 injection at the time of discectomy acts as a tissue protective agent improving cell viability and limiting matrix degradation and NO, although in vivo and human validation is required.

**Significance:** This study identifies potential mechanisms for post-discectomy-induced-degeneration and provides a proof of concept that biologic injection at the time of discectomy has the potential to augment current surgical practice.

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Fig. 1: Discectomy and TGF-β3 injection before culture a-c: discectomy performed on bovine IVD; d+e: A total of 20 μl TGF-β3 injection in several locations of the discectomy site. "X" mark injection sites; EP = endplate, NP = nucleus pulposus, AF = annulus fibrosus
**AF**

0.2 μg TGFβ3 injection after 7 day culture

![Graph showing gene expression analysis normalized to PBS injected controls.](image)

For the dose response screening IVDs were injected with 0.2 μg TGFβ3 in 100 μl PBS and cultured for 7 days. Increased expression of matrix proteins was observed which was significant for COL1 and MMP1. While no differences were observed for the inflammatory cytokine IL-1β, expression of TNFα decreased slightly (*=p<0.05; #p=0.09).
Fig. 3: Cell viability and dextran diffusion after discectomy. Cell viability after 6 (a) and day 19 (b) days of control injured and treated AF tissue (is = injury site) * = p < 0.01; c. Dextran injection after discectomy. Cracks in the matrix demarcated by * are due to the discectomy procedure. After 20 hours culture FITC labeled dextran remained within the tissue, localized close to the injected region.
Fig. 4: Aggrecan degradation and NO release after 19 day culture.  
a: aggrecan degradation products were analyzed in iAF extracts of control, injured, and treated IVDs by SDS page and western blotting using antibodies recognizing the aggrecan G1 domain (left) and the cleavage products of MMPs (DIPEN, center) and aggrecanase (NITEGE, right).  
b: total NO release into the media over 19 day culture (*=p<0.05).