Effects of Depth of Annular Injury and Tumor Necrosis Factor-alpha on Disc Degeneration and Pain

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Introduction: Development of an in-vivo animal discogenic pain model is a high research priority, which can provide insight into the pathophysiology of painful disc degeneration and serve as a screening tool for therapeutic intervention. Disc injury and inflammation may play an important role in discogenic pain, and our previous study demonstrated that annular puncture with intradiscal injection of tumor necrosis factor-alpha (TNFα) induced disc degeneration with painful behavior in an in-vivo rat model [1]. Annular injury is commonly adopted to induce degeneration in rodent lumbar discs, and more severe degeneration is observed following larger injuries with greater annular tissues damaged [2]. However, there is limited information about the depth of annular injury on disc degeneration and pain. Clinically, painful conditions can arise from both complete (i.e., deep) annular tears that expose the nucleus and lead to depressurization as well as incomplete (i.e., shallow) annular tears involving the outer annulus only. We hypothesize that the depth of annular injury will have distinct effects on pain and degeneration and the objective was to determine whether complete annular tear with intradiscal injection of TNFα induced more severe disc degeneration and painful behavior than incomplete annular tear in rat in-vivo.

Methods: All experimental procedures were approved and guided by the Institutional Animal Care and Use Committee. Thirty-six healthy, skeletally mature (4-5 months old) Sprague-Dawley rats were used, and were randomly divided into six groups (n=6): A) naïve, B) sham surgery, C) PBS_shallow, D) PBS_deep, E) TNFα_shallow, and F) TNFα_deep. Animals were operated under sterile conditions and general anesthesia. Peritoneal cavity was opened via midline abdominal incision, L3-4, L4-5 and L5-6 lumbar discs were visualized and identified using pelvic rim as an anatomic landmark. Discs were punctured using a 26-gauge needle with a depth of 1.5mm for incomplete annular tear and 3mm for complete annular tear, which was guided by a needle stopper. 2.5 μL of PBS or TNFα (0.25ng in 2.5ul) [3] was slowly injected into each disc. The abdominal wound was closed and the rats were monitored to ensure no serious impairments. Sham surgery without puncture or injection and naïve (without surgery) groups were included. Pain in rats was assessed weekly using hindpaw mechanical hyperalgesia tests with calibrated von Frey filament (0.6-2.4g). Severity of disc degeneration was determined using weekly radiographic disc height, postmortem magnetic resonance imaging (MRI) and histology. Six weeks after surgery, rats were euthanized. Lumbar spines were harvested, fixed, decalcified, embedded in paraffin, sectioned at 5µm and stained with Safranin-O/fast-green/hematoxylin for morphology and glycosaminoglycan (GAG) content. For statistical analysis, results of paw withdrawal threshold and disc height at each time point were normalized to pre-surgery, and compared using repeated measures ANOVA with least significant difference as post-hoc comparison to determine the effect of time.
Results: Withdrawal threshold of naive group did not show obvious changes throughout the 6 week experiment (p>0.05). Withdrawal threshold transiently decreased after sham surgery (p<0.05), but recovered after one week. Intradiscal injections induced continuous decreases in the threshold (p<0.05), with more obvious changes after TNF injections compared to PBS injections (p<0.05). However, intradiscal injections induced continuous decreases in disc heights (p<0.05), with the heights of disc after complete annular tear significantly smaller than those after incomplete injury. MRI showed similar changes. More obvious degenerative changes with decreased signal intensity were found in complete injured discs compared to incomplete injured discs. There was no herniation in any injected discs detected on histology or MRI. Normal disc morphology with organized annular lamellae with fibroblast-like annular cells and GAG-rich nucleus pulposus (NP) with notochordal NP cells was seen in both naive and sham groups. The annular puncture with injections mainly affected the annular morphology with disorganized annular lamellae and decreased GAG content and annular cells along the needle track. Complete annular injury induced more severe degeneration with further decreased GAG content, reduced disc height, more fibrous NP with decreased nuclear cells, and less distinct nuclear boundary.

Discussion: Annular puncture with intradiscal injection of TNFα or PBS induced behavioral and structural changes representative of discogenic pain with continuous and significantly increased hindpaw pain sensitivity, loss of disc height, reduced MRI intensity, and disrupted morphology. The severity of degeneration might be associated with depth of injury and complete annular injury was likely to injure NP, which exhibited a more fibrous NP with decreased nuclear cells compared to incomplete annular injuries. These degenerative changes might result from loss of nuclear pressure and reduced GAG, which might lead to loss of nucleus water content evidenced by reduced signal intensity in MRI, and decrease in disc height. Increased pain-sensitive behavior was observed in rats with degenerated discs, although appeared to be insensitive to puncture depth. Compared to PBS injections, TNFα injections further increased the painful behavior, which suggests an association between discogenic pain and inflammation. TNFα may play an important role in the initiation of discogenic pain by: 1) creating a pro-inflammatory state in the lumbar spine region; 2) promoting neurovascular ingrowth via up-regulations of nerve growth factor and vascular endothelial growth factor, as well as promoting breakdown of extracellular matrix which demonstrated to be an inhibitor of neurovascular ingrowth [4], and 3) up-regulating substance P, a neurotransmitter for transmitting nociceptive information.

Significance: The findings suggest that the extent of disc injury is the most important determinant of disc degeneration while the inflammatory state of the spine may be more relevant to discogenic back pain. These discogenic pain models with different severities of degeneration have significance for their use understanding mechanisms of discogenic pain and for screening future treatment modalities.