Inflammatory Kinetics And Efficacy Of Anti-inflammatory Treatments On Degenerated Human Nucleus Pulposus Cells

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Disclosures:

Introduction: Inflammation is a significant contributor to painful intervertebral disc (IVD) degeneration [1,2] and improved understanding of how the inflammatory cascade progresses in degenerated NP cells is required to inform future therapies. Anti-inflammatory therapies have been suggested as potential treatments to address the inflammatory component of IVD degeneration. However, multiple cytokines (IL-1β, IL-6, TNFα) have been suggested as key mediators in IVD pathology and it remains unknown which targets may be the most effective at reducing the overall inflamed environment. The aims of this study were to (1) assess how an inflammatory cascade develops following TNFα exposure, (2) explore the inter-cytokine kinetics via cytokine specific inhibition and (3) determine which anti-inflammatory therapy (IL-1Ra, anti-TNFα, anti-IL-6 and sodium pentosan polysulfate (PPS)) is most effective at reducing the overall inflamed environment. These aims were addressed through applying TNFα and anti-inflammatories in a 3D cell culture model and assessing the inflammatory response via ELISA and qPCR.

Methods: Human NP cells were isolated from 7 IVD samples (Thompson grade III-V) obtained from autopsy or surgery with IRB approval. NP cells were expanded in monolayer (5% CO2 37°C) using high glucose DMEM (10% FBS, 50ug/mL Ascorbic acid, 1% Pen/Strep). Cells (passage ≤ 4) were then suspended in alginate beads (2x10⁶ cells/mL). Cells were treated with TNFα for 72hrs prior to the application of one of the four drugs and taken down at 144hrs (72hrs after drug was added). This time course was chosen to simulate the clinical situation where anti-inflammatory therapies would treat IVDs following TNFα exposure, (2) explore the inter-cytokine kinetics via cytokine specific inhibition and (3) determine which anti-inflammatory therapy (IL-1Ra, anti-TNFα, anti-IL-6 and sodium pentosan polysulfate (PPS)) is most effective at reducing the overall inflamed environment.

Results: All cells remained viable throughout all experiments. Timecourse Study: TNFα induced increases in both protein and mRNA of all measured cytokines. All cytokines came to equilibrium within 72 hours however all at different magnitudes (72hrs - IL-8: 7938±2243pg/mL, IL-6: 1241±1146pg/mL IL-1β: 120±105pg/mL) (Fig 1B). Interestingly there was no observed lag between mRNA and protein expression. Negligible amounts of pro-inflammatory cytokines were detected in the basal control samples in both blocking studies (72hrs - IL-8: 166±111pg/mL, IL-6: 6.9±10pg/mL IL-1β: 2.6±3.9pg/mL) Same-time Blocking Study: For ELISA results (Fig 1C), anti-TNFα significantly inhibited the expression of IL-1β, IL-6 and reduced the amount of soluble TNFα. PPS significantly inhibited IL-1β and had a trend of decreased IL-6. The anti-IL-6 and IL-1Ra did not influence the expression of any cytokine following TNFα exposure. At 72 hours both anti-TNF and PPS had a trend of decreased IL-1β (p=0.1) and IL-6 (p=0.1) gene expression. Delayed Blocking Study: For ELISA results (Fig 1C), all the anti-inflammatories were much less effective at reducing the amount of soluble pro-inflammatory cytokines when they were introduced into a previously inflamed environment, with no drug having any significant changes. On the gene level both PPS & anti-TNF reduced IL-1 gene expression (p<0.05) and anti-TNF reduced TNFα gene expression (p<0.05).
Discussion: Multiple inflammatory cytokines are implicated in the pathogenesis of IVD degeneration however the kinetics and interactions between cytokines are unknown and are necessary to understand in order to develop and evaluate therapies. Timecourse Study: TNFα exposure induced the expression of all measured cytokines which reached equilibrium within 72 hours and all cells remained responsive as the concentration returned to the same levels even after a media change (Fig 1A). TNFα exposure substantially increased IL-6 and IL-8 compared to IL-1β and as both IL-6 & IL-8 are associated with pain [4,5] may suggest a role of how TNFα contributes to painful IVD degeneration. This study also confirms that native NP cells can contribute to the pathologic presence of inflammation within the IVD and suggest that once an inflamed environment develops it may be self-perpetuating. Blocking Studies: Multiple anti-inflammatory agents have been proposed as candidate therapies to address the elevated inflammation associated with IVD pathology. Both blocking studies showed that inhibition of IL-1 or IL-6, which has been shown to potentiate the effects of TNFα, did not influence the expression of any cytokine. However both PPS and anti-TNF inhibited IL-1β protein when added at the same time as TNFα and had a trend of reduced IL-1β gene expression when added in an inflamed environment. Results suggest that the timing of the intervention is critical, as anti-inflammatories only had a significant effect on protein when added at the same time as TNFα stimulation and not once an inflamed environment had developed (Fig 1C). This suggests that anti-inflammatories immediately following surgery may be successful at preventing an inflammatory cascade however a more aggressive therapy may be required for treating a chronically inflamed IVD.

Significance: Improved understanding of the kinetics and interactions between cytokines is required to better understand IVD pathology and to inform potential treatments. NP cells produce multiple pro-inflammatory cytokines and can contribute to the chronic inflammation associated with IVD degeneration. Use of a broad acting anti-inflammatory treatment or addressing the source of inflammation reduced the progression of the inflammatory cascade while anti-IL-6 and anti-IL-1 treatments had no effect. The timing of an anti-inflammatory intervention is critical to therapeutic success.

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Figure 1. A. Study design for Same and Delay study  
B. Timecourse: Changes in IL-1β, IL-8, & IL-6 over time in response to TNFα exposure  
C. ELISA Blocking Results: Changes in each cytokine concentration in response to different anti-inflammatories