In Vitro Investigation of Cytokine-Mediated Nucleus Pulposus Degeneration

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Motivation

- **Low back pain**
  - High prevalence: 25% of US population
  - Physically and financially detrimental

- **Limited treatment**
  - Aimed at alleviating painful symptoms
  - Incomplete understanding of biological mechanisms involved

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**Degeneration of lumbar intervertebral discs is strongly implicated as a cause of low back pain.**
Intervertebral Discs

- Spine consists of alternating:
  - Vertebrae
  - Intervertebral discs (IVDs)

- Function of IVDs
  - Transfer and distribute compressive loads
  - Permit spinal movement

http://www.spineuniverse.com/conditions/back-pain/anatomy-back-pain
Disc Anatomy

- Annulus Fibrosus (AF)
- Cartilaginous End Plates
- Nucleus Pulposus (NP)

http://www.chiropractic-help.com/L4-Lumbar-Spine.html
Nucleus Pulposus

- Pressurized gel
  - Randomly distributed network of collagen II
  - High hydrated extracellular matrix rich in proteoglycans

- Mechanical Function
  - In compression confined peripherally by AF
  - Generating a region of hydrostatic pressure

Human Disc Degeneration

- Degeneration starts in the NP
- Compositional Changes
  - Loss of glycosaminoglycans (GAG)
  - Loss of water
- Impaired mechanical function
  - Reduced NP pressure
  - Altered motion segment stiffness
- NP changes initiate a cascade expanding to other structures
  - Loss of disc height
  - Inward bulging of AF
  - Formation of tears

Cytokine-Mediated Matrix Degradation

- Pro-inflammatory cytokines
  - Interleukin-1 beta (IL1β)
  - Tumor Necrosis Factor alpha (TNFα)

- Naturally occurring inhibitors of cytokines
  - Interleukin-1 receptor antagonist (IL1ra)
  - Soluble TNF receptor 1 (sTNFR1)

- In IVD degeneration, up-regulation of cytokines, no matched increase of inhibitors
  - Increases in catabolic enzymes: MMP3, MMP13, ADAMTS4
  - Decreases in NP proteins: aggregan and collagen II
Previous Studies

- Association of IL1β with IVD

- Association of TNFα with IVD

Debate: roles of these cytokines in initiating NP matrix changes

Gap: what is the functional significance of these matrix changes?
Objective

Use an in-vitro NP model to investigate:

1. Effects of IL1β and TNFα on composition and mechanical function

2. Capacity of IL1ra and sTNFR1 to mitigate cytokine-mediated changes
Methods

- **Cell Isolation**
  - Mature NP cells isolated from bovine caudal discs

- **NP Constructs and Treatment**
  - NP cells seeded at 20X10^6 cells/ml in agarose gels (4mm diameter X 2.254mm thick)
  - Precultured 6 weeks with transforming growth factor beta 3 (TGF-β3) before treatment

<table>
<thead>
<tr>
<th>IL1 Treatment Groups (n=7)</th>
<th>TNF Treatment Groups (n=7)</th>
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</thead>
<tbody>
<tr>
<td>IL1β, 10ng/ml</td>
<td>TNFα, 10ng/ml</td>
</tr>
<tr>
<td>IL1β, 10ng/ml + IL1ra, 100ng/ml</td>
<td>TNFα, 10ng/ml + TNFR1,100ng/ml</td>
</tr>
<tr>
<td>IL1ra, 100ng/ml</td>
<td>TNFR1, 100ng/ml</td>
</tr>
<tr>
<td>Control (no treatment)</td>
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</tr>
</tbody>
</table>
Histology

- **Embedding**
  - Samples fixed in 4% paraformaldehyde and dehydrated in graded series of ethanol
  - Embedded in paraffin
  - Sectioned at 7 μm-thickness from middle

- **Staining**
  - Alcian Blue (AB): GAG
  - Picrosirius Red (PR): collagen
Mechanical Testing

- Confined compression
  - to replicate physiological conditions of NP

- Device
  - Acrylic chamber fixed above porous platen
  - Impermeable ceramic indenter for applying compression

- Tests
  - Static preload (0.02N for 500s): equilibrated thickness
  - Stress relaxation test: 10% strain applied at 0.05%/s, then relaxation to equilibrium for 10min

- Calculations
  - Aggregate modulus ($H_A$): final stress/applied strain
  - Hydraulic permeability ($k_0$): linear biphasic theory
Biochemical Analysis

- **Preparation**
  - Wet and dry weights of samples
  - Papain digestion for dried samples

- **Assays**
  - DMMB (1,9-dimethylmethylene blue dye-binding) assay: GAG
  - OHP (orthohydroxyproline) assay: collagen
    - First acid hydrolysis of sample digests
Results - Histology

- **GAG (AB):**
  - Uniformly distributed and intense
- **Collagen (PR):**
  - More diffuse, pericellular and intercellular

Histology staining for functionally mature constructs.
Results - Mechanics

- **IL1 Treatment Groups**
  - IL1β: 33% decrease in $H_A$ and 41% increase in $k_0$
  - IL1β +IL1ra and IL1ra only: no significant difference

- **TNF Treatment Groups**
  - No changes in $H_A$ and $k_0$

A. aggregate modulus,
B. hydraulic permeability (*p<0.05)
Results - Biochemical Analysis

- **GAG:**
  - IL1β: 27% decrease relative to untreated controls
  - IL1β + IL1ra and IL1ra only: no significant difference from controls, significantly greater than IL1β only
  - All TNF groups: no significant difference
- **Collagen:** similar trends as GAG, no significant differences

![Bar chart showing GAG and Collagen content](Image)
Conclusions

- IL1β plays a more direct role than TNFα
  - Mechanical and biochemical analyses: significant effects for IL1 treatment groups, not TNF groups
  - Short-term exposure to IL1β induced matrix changes that are functionally significant

- Significant inhibitory effects place IL1ra as a key therapeutic agent
  - IL1ra can effectively prevent NP matrix changes and associated functional changes induced by IL1β

- Results are clinically significant for developing novel treatment approaches for IVD degeneration
Future Work

- Investigate gene expression
  - Down-regulated anabolic genes and up-regulated catabolic genes associated with degeneration
  - Quantify changes in catabolic enzyme activity and matrix synthesis following cytokine exposure

- Develop biodegradable polymeric microspheres to deliver therapeutic agents

- Evaluate therapeutic agents in an in-vivo model of disc degeneration
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