Evaluating an Interleukin-1β Injection to Induce Degeneration in the Rat Lumbar Spine

Alta Berger
George Washington University
Amy Orlansky, Dawn M. Elliott
McKay Orthopaedic Research Laboratory
University of Pennsylvania

SUNFEST Symposium
August 7th, 2008
The Intervertebral Disc

- **Composition:**
  - **Nucleus Pulposus**
    - Gelatinous center, provides osmotic pressure for absorption of force
  - **Annulus Fibrosus**
    - Highly organized fibers that encircle the NP

Disc Degeneration

• Begins in the NP
  – ↓ in water content
  – ↓ in GAG content

• Spreads to AF
  – Disorganization
  – Tears

(Adams et al. 2002)
Previous Studies

- ChABC used to decrease GAG content
- Corresponded to loss of mechanical function
Interleukin-1

• Naturally occurring cytokine linked to cartilage degradation
• Shown to be produced by both degenerate and non-degenerate discs
• Linked to matrix degrading enzymes, decrease in production of proteoglycans
Summer Goal

• Create *in-vivo* rat model of intervertebral disc degeneration brought on by IL-1
• Hypothesis:
  IL-1 will cause decrease in GAG content and a loss of mechanical function
Method

- 3 groups: IL-1, PBS Sham, Control (1 μL injections)
- 3 discs injected in each rat
- Euthanized 1 or 4 weeks post injection
- Motion segments harvested, kept at -20°C until testing
Biochemistry

- Discs isolated from motion segment
- Customized punches used to separate NP, IAF, and OAF
- Spectrophotometer used to determine GAG content based on the color metric scale from DMMB assay
Mechanical Testing

- Axial Compression-Tension
  cyclic testing: 20 cycles of 4.5 N compression to 3 N Tension
- 45 minute Creep test
- Data from final cycle analyzed using trilinear fit model through MATLAB
Results - Water Content

Water Content in the NP

![Bar chart showing water content in IL-1 and PBS conditions for 1 Week and 4 Week](chart.png)
Results - Water Content

Water Content in the AF

<table>
<thead>
<tr>
<th></th>
<th>Water Content (%)</th>
<th>1 Week</th>
<th>4 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAF IL-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OAF IL-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAF PBS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OAF PBS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results - GAG content

GAG Content in the NP
(per Wet Weight)

<table>
<thead>
<tr>
<th>GAG/WW (ug/mg)</th>
<th>1 Week</th>
<th>4 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results - GAG Content

GAG content in the AF of Rat Discs
(per Wet Weight)

GAG/WW (ug/mg)

<table>
<thead>
<tr>
<th></th>
<th>IAF</th>
<th>OAF</th>
<th>IAF</th>
<th>OAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Week</td>
<td>23</td>
<td>10</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>4 Week</td>
<td>27</td>
<td>18</td>
<td>21</td>
<td>8</td>
</tr>
</tbody>
</table>

PBS
Results - Mechanics

Load Displacement 4 Week Post Injection

Displacement (mm)

Force (N)

IL-1_1
IL-1_2
PBS_1
PBS_2
Results - Mechanics

Mechanics Data for Rat Lumbar Disc
4 weeks post injection

Displacement (mm)

NZ Length

ROM

IL-1  PBS
Results - Mechanics

Neutral Zone Stiffness for Rat Lumbar Disc 4 Weeks Post Injection

Stiffness (N/mm)

IL-1

PBS
Results - 1 week Histology

IL-1

PBS

Control
Results - 4 week Histology

IL-1

PBS

Control
Results

- Biochemical results correspond to mechanics
- Recovery of GAG
- % difference of GAG content in IL-1 vs. PBS discs per wet weight
  - 1 week: 8.6265%
  - 4 week: 51.2315%
Increase in GAG

- Possible that the presence of IL-1 caused an increase in IL-1 RA
  - Further regulation
- If IL-1 acts as foreign substance, cells may produce antibodies against it to prevent further attack
- Future studies: observe levels of IL-1 RA in addition to IL-1 in the disc
Challenges

• Difficulties with mechanical testing led to loss of 1 week data
• Small study (n=2)
• Discs susceptible to tears during dissection and sectioning
Future Work

• Develop method to provide lasting effect of IL-1: released over time
• Observe levels of IL-1 RA
• Monitor *activity* of IL-1 in the disc
• Larger study
Results: Interleukin-1b

GAG Content in the Rat Disc (5 Days Post Surgery)

- Control (n=3)
- IL-1b: 200 ng (n=3)
Results: Interleukin-1β

Hydroxyproline Content in Rat Disc (5 Days Post-Surgery)

- Control (n=3)
- IL-1β: 200 ng (n=3)

Bar chart showing hydroxyproline content in NP, IAF, and OAF with control and IL-1β treatments.
Results: Interleukin-1b

Water Content in Rat Discs
(5 Days Post-Surgery)

Control (n=3) vs. IL-1b: 200 ng (n=3)

- NP
- IAF
- OAF

H2O Content (%)
Future Work

• Increase sample size to look at impact of IL-1 on the disc at short, medium, and long time points

• Establish temporal inter-relationships between mechanics, biochemistry, molecular and cellular events as a results of IL-1beta in the rat disc