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

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The Intervertebral Disc

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

- Begins in the NP
  - ↓ in water content
  - ↓ in GAG content
- Spreads to AF
  - Disorganization
  - Tears

(Adams et al. 2002)
- 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

http://www.paulnoll.com/China/Zodiac/zodiac-rat-pic.gif
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

Water Content (%)

1 Week

4 Week

IL-1

PBS

1 Week

4 Week
Results - Water Content

Water Content in the AF

<table>
<thead>
<tr>
<th>Sample</th>
<th>Water Content (%)</th>
<th>IL-1</th>
<th>PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAF</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OAF</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAF</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OAF</td>
<td>60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- 1 Week
- 4 Week
Results - GAG content

GAG Content in the NP
(per Wet Weight)

<table>
<thead>
<tr>
<th>GAG/WW (μg/mg)</th>
<th>IL-1</th>
<th>PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Week</td>
<td>28</td>
<td>32</td>
</tr>
<tr>
<td>4 Week</td>
<td>46</td>
<td>33</td>
</tr>
</tbody>
</table>

IL-1 PBS
Results - GAG Content

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

GAG/WW (ug/mg)

<table>
<thead>
<tr>
<th></th>
<th>1 Week</th>
<th>4 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OAF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Week
4 Week
Results - Mechanics

Load Displacement 4 Week Post Injection

![Graph showing load displacement for 4 week post injection with different groups: IL-1_1, IL-1_2, PBS_1, PBS_2.](image)
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)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Stiffness</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td>18</td>
</tr>
<tr>
<td>PBS</td>
<td>12</td>
</tr>
</tbody>
</table>
Results - 1 week Histology

IL-1

Control

PBS
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)

GAG/Dry Weight (ug/mg)

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

NP
IAF
OAF
Results: Interleukin-1b

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

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

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

- Control (n=3)
- IL-1b: 200 ng (n=3)
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
Thank You

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