THE EFFECTS OF EXERCISE AND INJURY ON CHONDROITIN SULFATE AND KERATAN SULFATE CONTENT IN EQUINE SYNOVIAL FLUID

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Introduction: Our previous work, as well as that of others, has shown that injury-mediated activation of cartilage matrix degradation in adult horses results in proteolytic fragmentation of aggrecan, which in turn results in the release of chondroitin sulfate (CS) and keratan sulfate (KS) substituted degradation fragments into the synovial fluid and serum. We hypothesized that measurement of these degradation products by fluorophore-assisted carbohydrate electrophoresis (FACE) may provide useful markers of acute joint injury.

Methods: By using FACE, CS and KS content were measured in equine carpal synovial fluid. Specifically, chondroitinase ABC/ACII products - non-sulfated chondroitin (?diOS), chondroitin-6-sulfate (?di6S), and chondroitin-4-S (?di4S) were quantitated. Additionally, the keratanase II/endo-β-galactosidase products of KS - the mono- and disulfated disaccharides, galβ1,4glcNAc6S (monoS) and galβ1,4glcNAc6S (diS) were measured.

Four groups of horses were compared: (1) racehorses undergoing arthroscopic surgery, (2) age-matched horses with chronic osteoarthritis (OA), (3) age-matched, normal unexercised controls, (4) treadmill trained horses, both before and after stress testing. The stress test was a period of maximal exercise in which treadmill speed was incrementally increased until the horse could no longer maintain position on the treadmill. The protocol was approved by the Institutional Animal Care and Use Committee.

Results: Significant differences were observed between groups for a number of the GAG disaccharides, most notably ?diOS, ?di4S, and DiS (Table 1). ?diOS and ?di4S concentrations are plotted in Fig 1.

Table 1 – Glycosaminoglycan disaccharide concentrations (mean ± SD) in synovial fluid (SF)

<table>
<thead>
<tr>
<th>Value</th>
<th>Normal</th>
<th>Arthroscopy</th>
<th>OA</th>
<th>Treadmill</th>
</tr>
</thead>
<tbody>
<tr>
<td>?diOS (µg/ml of SF)</td>
<td>(n=15)</td>
<td>(n=19)</td>
<td>(n=7)</td>
<td>(n=15)</td>
</tr>
<tr>
<td>?di6S</td>
<td>3.6±2.6*</td>
<td>7.3±5.5*</td>
<td>6.8±5.5*</td>
<td>2.2±0.6*</td>
</tr>
<tr>
<td>?di4S</td>
<td>11.5±4.5*</td>
<td>16.7±9.7*</td>
<td>20±12*</td>
<td>11.3±4.6*</td>
</tr>
<tr>
<td>MonoS</td>
<td>1.7±0.7*</td>
<td>1.7±1.2*</td>
<td>1.5±1.2*</td>
<td>2.1±0.8*</td>
</tr>
<tr>
<td>DiS</td>
<td>0.9±0.6*</td>
<td>1.2±0.4*</td>
<td>1.8±1.0*</td>
<td>2.2±1.2*</td>
</tr>
<tr>
<td>MonoS / DiS</td>
<td>2.3±1.3*</td>
<td>1.6±2.7*</td>
<td>0.7±0.3*</td>
<td>1.8±2.3*</td>
</tr>
</tbody>
</table>

Comparisons between groups were made by Kruskal-Wallis non-parametric test. Pre-stress and post-stress treadmill data were compared using the Wilcoxon signed rank test. Arthro – arthroscopy.

Conclusions: Our most significant findings from this data are the differences between the arthroscopic synovial fluids and those from the treadmill horses. ?diOS and ?di4S were significantly higher in the arthroscopic fluids than in the treadmill fluids. We found in our previous work that the cartilage from arthroscopic joints had higher ?diOS and ?di4S than did age-matched controls, and therefore it appears that these changes in the cartilage are reflected in the synovial fluid, as we had predicted. Presumably, fibrillation and fragmentation of damaged cartilage in horses with chip fractures would more readily allow the release of the CS fragments in greater quantities, thus accounting for the higher concentrations of ?diOS and ?di4S in the SF. As a result, total CS was also significantly different between these groups. It would seem logical that horses in a regular exercise program, as these treadmill horses were, make a more appropriate

Fig 1 – Comparison of synovial fluid (SF) concentrations of ?diOS (upper) and ?di4S (lower) between groups of horses.

control group for the arthroscopic cases than would normal, sedentary horses, as are often used for these studies.

We found the uniformity of ?diOS data obtained from the treadmill horses very interesting. It is possible that this uniformity comes from increased clearance of CS from the joint associated with exercise. However, increased joint clearance would not explain the KS data from treadmill horses, because they had a tendency to higher KS values in the synovial fluid (significantly different from unexercised controls).

Perhaps exercise has a metabolic effect which causes the differences seen in CS and KS concentrations in synovial fluid. We were unable to determine the underlying cause from our study.

The only significant difference noted between synovial fluids from arthroscopic cases and the normal, pasture-maintained horses was that the ?di4S was significantly higher in the arthroscopic fluid.

It is interesting to note that no significant differences were found on comparisons made between arthroscopic and osteoarthritic synovial fluids. We had expected that the more acute injuries associated with the arthroscopic cases may have shown higher concentrations of ?diOS and ?di4S. We thought that the chronicity of the osteoarthritic cases may have allowed time to return to more normal sulfation patterns.

Although the treadmill horses tended to have higher concentrations of mono- and disulfated-KS than the other groups, the only significant differences seen were that the pre-stress tested joints had significantly higher monoS and total KS than the normal, unexercised horses.

However, the monoS/diS ratio was significantly higher in the normal horses than in osteoarthritic horses. Among treadmill horses, observed differences of monoS and KS values before and after stress testing were small, but significantly different.

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