Changes in Biochemical Markers and Prediction of Efficacy of Intraarticular Hyaluronan Injection for Knee Osteoarthritis

Masahiro Hasegawa, Yutaka Nakoshi, Masaya Tsujii, Akihiro Sudo, Hiroyuki Masuda, Toshimichi Yoshida, Atsumasa Uchida

1Department of Orthopaedic Surgery, Mie University Graduate School of Medicine, Tsu, Mie, Japan; 2Seikagaku Corporation, Tokyo, Japan; 3Department of Pathology & Matrix Biology, Mie University Graduate School of Medicine, Tsu, Mie, Japan

masahase@clin.med.mie-u.ac.jp

Introduction: Articular cartilage is composed of chondrocytes embedded in an extracellular matrix of principally type II collagen and proteoglycan aggrecan molecules. In osteoarthritis (OA), the release of degraded aggrecan molecules, including chondroitin sulfate (CS) and keratan sulfate (KS), from the matrix into synovial fluid (SF) can be detected and reflective of aggrecan turnover. Adult human articular cartilage mainly contains chondroitin 6-sulfate (C6S), which is converted to chondroitin 4-sulfate (C4S) in OA cartilage. In contrast, the synovium, meniscus, and ligaments predominantly contain the C4S isomer. KS is found almost exclusively in cartilage aggrecan [1]. Tenascin-C (TN-C) is a hexameric glycoprotein component of the extracellular matrix. TN-C is up-regulated in many pathologic adult conditions, including tumorigenesis, regeneration, and inflammation. We reported TN-C could be a useful biochemical marker for OA [2] and rheumatoid arthritis (RA) [3].

The intra-articular injection of hyaluronan (HA) has been extensively used in the treatment of OA. In this study, we observed whether repetitive intra-articular injections of HA in patients with OA induced significant changes in SF levels of biochemical markers, including C6S, C4S, KS, and TN-C. In addition, we investigated the possibility of predicting the effectiveness of HA based on levels of these biochemical markers in the SF.

Materials and Methods: Twenty-eight patients fulfilling the American College of Rheumatology criteria of knee OA underwent 5 weekly intra-articular injections of HA. The cohort consisted of 19 women and 9 men whose mean age and body mass index were 76.7 years (range, 62-88 years) and 23.5 kg/m² (range, 17-31 kg/m²), respectively. No concomitant steroid therapy or nonsteroidal anti-inflammatory drugs were administered. Patients were divided into 4 groups based on radiographic grading of the OA severity described by Kellgren and Lawrence. Two independent readers blinded to the source of the data graded the knees. Two patients were grade 1, 12 were grade 2, 9 were grade 3, and 5 were grade 4. All patients gave informed consent, and this study was approved by the local ethics committee. Patients were treated with 5 weekly intra-articular injections of 1% HA solution (Artz, Seikagaku Corporation, Tokyo, Japan). SF samples were collected before each HA injection.

Knee pain was measured on a 100-mm visual analog scale (VAS; 0 mm = no pain, 100 mm = worst imaginable pain) before the first injection and after 5 weekly injections of HA. C6S, C4S, and KS in the SF were measured by high performance liquid chromatography. Levels of large-subunit TN-C were determined using an enzyme-linked immunosorbent assay. Continuous variables were compared by the Student t-test or Mann-Whitney U test. Correlations between levels of biochemical markers before injection and improvement of VAS were also estimated. The Kruskal-Wallis test was used to determine the difference between radiographic stages of OA and improvement of VAS.

Results: A significant reduction in VAS was recorded after 5 weekly injections (27 ± 18 mm) compared with baseline (56 ± 19 mm, P < 0.001). The concentrations of C6S, C4S, and KS were significantly decreased after 5 weekly HA injections (Table 1), however, TN-C levels showed no changes before or after HA injections. Table 1. Concentration of biochemical markers before HA injection and after 5 weekly injections.

A significant inverse correlation was observed between the TN-C levels before HA injection and improvement of VAS after 5 weekly HA injections (R = -0.405, P = 0.035, Figure 1). The concentrations of C4S before injection also showed an inverse correlation with improvement of VAS after 5 weekly HA injections (R = -0.409, P = 0.034, Figure 1). In contrast, no significant correlation was seen between C6S and KS levels before injection and improvement of VAS after 5 weekly HA injections (C6S: P = 0.183; KS: P = 0.803). Radiographic stages of OA had no significant relation with improvement of VAS (P = 0.201).

Discussion: One of the most obvious uses of biochemical markers is their potential to shed light on the effects of the treatment on the metabolism of joint tissue. Based on changes in biochemical marker levels after HA injections, the present study showed that catabolic markers, including C6S, C4S, and KS, decreased after HA injections.

The present study is the first, to our knowledge, to show some prediction of the effectiveness of HA injections for knee pain. Low levels of TN-C and C4S at baseline were associated with decreasing pain. Previous studies revealed that TN-C levels of SF were elevated in patients with advanced OA. In addition, previous studies have shown that levels of TN-C and C4S were higher in patients with RA compared with OA [3, 4]. It is possible that the proliferated synovium, as well as degenerated cartilage, accelerates the release of C4S into the SF. Our findings suggest that HA injections are effective for knees with low levels of TN-C and C4S, reflecting an early stage of OA and limited synovitis. Joint fluid analysis may provide useful information about the prediction of the efficacy at the time of the first injection of HA.


Table 1. Concentration of biochemical markers before HA injection and after 5 weekly injections.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Before</th>
<th>After</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6S (mg/l)</td>
<td>9.1 ± 3</td>
<td>5.5 ± 2.5</td>
<td>0.007</td>
</tr>
<tr>
<td>C4S (mg/l)</td>
<td>12.7 ± 7</td>
<td>10.8 ± 6.6</td>
<td>0.044</td>
</tr>
<tr>
<td>KS (mg/l)</td>
<td>9.5 ± 3</td>
<td>3.5 ± 3.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure 1. Correlation between synovial fluid levels of TN-C and C4S before HA injection and improvement of VAS after 5 weekly injections.