Changes in biochemical markers and prediction of effectiveness of intra-articular hyaluronic in patients with knee osteoarthritis

M. Hasegawa M.D., Ph.D., Y. Nakoshi M.D., M. Tsujii M.D., Ph.D., A. Sudo M.D., Ph.D., H. Masuda M.S., T. Yoshida M.D., Ph.D. and A. Uchida M.D., Ph.D.

Department of Orthopaedic Surgery, Mie University Graduate School of Medicine, Mie, Japan
Seikagaku Corporation, Tokyo, Japan
Department of Matrix Biology and Pathology, Mie University Graduate School of Medicine, Mie, Japan

Summary

Objective: Intra-articular injection of hyaluronic (HA) is frequently used to treat knee osteoarthritis (OA). We studied whether HA injections induced significant changes in levels of biochemical markers in synovial fluid (SF). In addition, we investigated the possibility of predicting the effectiveness of HA based on these biochemical markers.

Methods: Twenty-eight patients with knee OA underwent five weekly intra-articular injections of HA. Knee pain was measured on visual analog scale (VAS) before and after the five injections. Levels of biochemical markers, including chondroitin 6-sulfate (C6S), chondroitin 4-sulfate (C4S), keratan sulfate (KS), and tenascin-C (TN-C), were determined before and after the five injections. Correlations between the biochemical markers before HA injection and the improvement of VAS after the five injections were evaluated.

Results: After HA injections, levels of C6S, C4S, and KS decreased significantly. Inverse correlations were observed between the levels of TN-C and C4S before HA injection and improvement of VAS after the five injections. In contrast, no significant correlation was seen between levels of C6S and KS before injections and improvement of VAS after the five injections.

Conclusion: The reduction in C6S, C4S, and KS levels after HA injections reflects that HA could help maintain normal cartilage metabolism. Our findings suggest that HA injections are effective in patients whose knees contain low levels of TN-C and C4S, reflecting an early stage of OA and limited synovitis.

Key words: Hyaluronic, Knee osteoarthritis, Biomarker, Synovial fluid.

Introduction

Osteoarthritis (OA) is one of the most prevalent diseases in the aging population. This disease is characterized by changes in cartilage, subchondral bone, and synovium. Secondary synovitis resulting from the breakdown products of cartilage and bone matrix likely modifies the symptoms of OA. Articular cartilage is composed of chondrocytes embedded in an extracellular matrix of principally type II collagen and proteoglycan aggrecan molecules. The release of degraded aggrecan molecules containing glycosaminoglycans, including chondroitin sulfate (CS) and keratan sulfate (KS), from the matrix into synovial fluid (SF) can be detected and levels of these two biochemical markers are 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. Cartilage markers are divided into markers of synthesis and catabolism. Typical markers of catabolism reflect the destruction of cartilage. Catabolic markers include aggrecan molecules, CS, and KS. It is not clear whether these markers reflect degradation of mature resident proteoglycan or of newly synthesized molecules.

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 that TN-C levels of the SF were significantly correlated with radiographic progression of knee OA. Further, we have recently shown that SF levels of TN-C were higher in patients with rheumatoid arthritis (RA) compared with patients with OA. TN-C could thus be a useful biochemical marker for OA and RA.

The intra-articular injection of hyaluronic (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.
cohort consisted of 19 women and nine men whose mean age and body mass index (BMI) were 76.7 years (range, 62–88 years) and 23.5 kg/m² (range, 17.3–31.1 kg/m²), respectively. Most of the patients have no occupation with low activity. No concomitant steroid therapy or nonsteroidal anti-inflammatory drugs were administered. Patients were divided into four 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, nine were grade 3, and five were grade 4. All patients gave informed consent, and this study was approved by the local ethics committee.

Patients were treated with five weekly intra-articular injections of 1% HA (molecular weight about 900,000 Da) solution with a dosage of 2.5 ml/injection (Artz, Seikagaku Corporation, Tokyo, Japan). SF samples were collected before each HA injection. The SF was centrifuged at 15,000 x g for 15 min and the supernatants were stored at −80°C until analyzed.

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 five weekly injections of HA.

MEASUREMENT OF BIOCHEMICAL MARKERS

C6S and C4S in the SF were measured by high performance liquid chromatography (HPLC) as described previously. The SF samples were diluted 10-fold and treated by a series of digestions with chondroitinase ABC and chondroitinase AC-II (Seikagaku Corporation, Tokyo, Japan). The chondroitinase digestions produce the unsaturated disaccharides, 6-di-6S and 6-di-4S, from the structure of C6S and C4S in the CS chain. After ultrafiltration of the digested solutions, the levels of 6-di-6S and 6-di-4S in the filtrates were analyzed, and the area of the peak corresponding to each unsaturated disaccharide was calculated. KS levels were determined by HPLC according to the method by Yamada et al. Briefly, the SF samples were diluted 10-fold and treated with keratanase II (Seikagaku Corporation) to be digested to two disaccharide isomers, namely β-galactosyl-(1-4)-6-sulfo-N-acetylgalactosamine (L2) and β-6-sulfo-galactosyl-(1-4)-6-sulfo-N-acetylgalactosamine (L4). Then, concentrations of these disaccharide isomers were determined and the sum of these levels was considered the KS level.

The levels of TN-C correlated with C6S (P = 0.713, P < 0.001), but not between KS and C4S levels (R = 0.087, P = 0.653). No significant correlations between VAS and levels of biochemical markers were identified.

Table I

<table>
<thead>
<tr>
<th>SF levels of C6S, C4S, KS, and TN-C before HA injection (week 0) and after five weekly injections (week 5)</th>
<th>Week 0</th>
<th>Week 5</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6S (nmol/ml)</td>
<td>61.2 ± 35.8</td>
<td>52.8 ± 25.3</td>
<td>0.012</td>
</tr>
<tr>
<td>C4S (nmol/ml)</td>
<td>19.1 ± 6.7</td>
<td>17.8 ± 6.1</td>
<td>0.044</td>
</tr>
<tr>
<td>KS (μg/ml)</td>
<td>6.1 ± 3.1</td>
<td>5.2 ± 2.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TN-C (ng/ml)</td>
<td>37.4 ± 53.1</td>
<td>39.0 ± 58.1</td>
<td>0.411</td>
</tr>
</tbody>
</table>

Results are mean ± standard deviation.

CHANGES IN BIOCHEMICAL MARKERS AFTER HA INJECTIONS

The concentrations of C6S, C4S, and KS were significantly decreased after five weekly HA injections (Table I). TN-C levels showed no changes before or after HA injections (Table I).

CORRELATION BETWEEN BIOCHEMICAL MARKERS BEFORE HA INJECTION AND IMPROVEMENT OF VAS

A significant inverse correlation was observed between the TN-C levels before HA injection and improvement of VAS after five weekly HA injections (Fig. 1). The concentrations of C4S before injection also showed an inverse correlation with improvement of VAS after five weekly HA injections (Fig. 1). In contrast, no significant correlation was seen between C6S and KS levels before injection.
and improvement of VAS after five 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

Many randomized controlled studies have confirmed that intra-articular injections of HA can decrease knee pain in patients with OA \(^{18-20} \). These studies showed that the efficacy of HA treatment was superior to placebo, and the safety of HA treatment was also confirmed \(^{18-21} \). HA behaves as a viscous liquid at low shear rates and as an elastic solid at high shear rates. In addition to the rheological functions, HA has a variety of effects on cells in vitro. It inhibits prostaglandin \( E_2 \) synthesis induced by interleukin-1 (IL-1) and protects against proteoglycan release and cytotoxicity induced by oxygen-derived free radicals, IL-1, and matrix metalloproteinases \(^{22} \). HA induces IL-1, tumor necrosis factor \( \alpha \), and insulin-like growth factor 1 messenger RNA transcript expression that are blocked by monoclonal antibodies to the HA receptor CD 44 in monocyte \(^{23} \). In cartilage, HA suppresses cartilage matrix degradation \(^{24} \). HA has been shown to increase the synthesis of proteoglycans and collagen \(^{25} \). In animal models, studies attempting to ascertain whether intra-articular injections of HA modify structural damage in the OA joint have produced conflicting results. Numerous studies have shown that HA injections prevent cartilage degradation and release of proteoglycans \(^{26-30} \). HA has a positive effect on the maintenance of cartilage matrix integrity during the development of OA as well as on the reduction of synovitis \(^{31} \). However, Smith et al. \(^{32} \) showed a striking reduction in proteoglycan concentration in articular cartilage after HA injections in an OA model. This finding raises the concern that HA treatment could suppress the synthesis of proteoglycans and accelerate joint damage. There is an increasing interest in structure/disease modification as the goal in OA treatment, however, we have no universally proven structure-/disease-modifying interventions \(^{33} \).

A significant correlation between C6S and KS levels, but not between KS and C4S levels has been reported by others \(^{34} \) and was confirmed in the present study. This correlation is interesting, as C6S and KS are most concentrated in healthy adult human articular cartilage. In contrast, in OA cartilage, there is an increased level of C4S and a decreased level of C6S and KS. The proteoglycan fragments observed in OA SF have characteristics of degradation products of proteoglycan aggrecan molecules that are released from the original resident matrix. Thus, the disease process may be characterized by a continuing attack on the original resident molecules. The reduction in glycosaminoglycan levels in severe disease is probably associated with suppression of cartilage matrix turnover and the loss of residual cartilage, as a decrease in C4S levels as well as C6S levels is also observed at this stage \(^{35,36} \).

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. Several previous studies have studied SF markers in patients treated with HA injections. Creamer et al. \(^{37} \) reported that a decrease in KS levels occurred in knees treated with HA, although their findings did not reach statistical significance. Kobayashi et al. \(^{38} \) showed that SF levels of C4S and C6S were significantly decreased after five weekly intra-articular injections of HA. The level of intact aggrecan was stable during the series of HA injections in this study. This means that the level of degraded aggrecan alone was reduced in the SF by HA injections. The reduction in levels of C6S and KS after HA injections reflects that HA could inhibit the release of proteoglycans from degenerated cartilage, down-regulate the synthesis of proteoglycans for the process of cartilage repair, and maintain normal cartilage metabolism \(^{39,40} \). Smith et al. \(^{32} \) found favorable effects of HA treatment on suppressing proteoglycan synthesis after HA injections in an OA model.

Changes in biochemical markers during the course of HA treatment can help to determine the effectiveness of treatment. Because joint puncture is an invasive procedure, and has a risk of complications, including infection \(^{39} \), determining potential nonresponders can lead to more efficient use of HA. Only one previous study approached the prediction of the effectiveness with HA injections for the treatment of knee OA, and results showed positive correlations between levels of C6S and aggrecan (molecules with HA-binding ability and a KS side chain) before HA injection and the improvement of clinical scores after injections \(^{41} \). Improvement of clinical symptoms after initiation of HA injection can be predicted by measurement of the fragments derived from aggrecan. The authors concluded that HA injections were effective for knees with high levels of aggrecan fragments, reflecting an early stage of OA in keeping with residual cartilage and chondrocyte metabolic activity. However, the markers had no significant relation with improvement of VAS. The present study is the first, to our knowledge, to show some prediction of the effectiveness of HA injections for knee pain. However, limitations of this study include a small sample size, limited follow-up period, and absence of a control group.

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, studies have shown that levels of TN-C and C4S were higher in patients with RA compared with OA \(^{12,16} \). 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. Since blood or urine would have much wider application, further studies are needed to find blood or urine markers to predict HA effectiveness before the first injection.

Conflict of interest statement

No benefits or funds were received in support of this study.

References


