

Intraarticular Injection of High Molecular Weight Hyaluronan for Osteoarthritis of the Knee — Prediction of Effectiveness with Biological Markers

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ABSTRACT. *Objective.* The possibility of predicting the effectiveness of intraarticular injection of high molecular weight hyaluronan (HA) was investigated using biological markers.

Methods. In 32 patients with osteoarthritis (OA) of the knee, 38 knees were treated with HA injection, and the clinical symptoms were evaluated using the Japanese Orthopaedic Association (JOA) score and pain visual analog scale (VAS). The concentrations of chondroitin 6-sulfate, 4-sulfate (C6S, C4S), and aggrecan were measured in synovial fluid collected at the time of initiation of injection. The relationship between the biological markers and the improvement of clinical symptoms after injection for 1 month was investigated.

Results. C6S/C4S and concentration of aggrecan decreased after injection, although these decreases were not significant. Positive correlations were noted between the concentrations of C6S and aggrecan before HA injection and the improvement of the JOA score after injection; however, radiological OA stage had no significant relation with improvement both of the JOA score and VAS.

Conclusion. It has been reported that the concentration of aggrecan-derived fragments in synovial fluid decreases with advancement of the OA stage, reflecting decreases in the amount of residual cartilage and suppression of chondrocyte metabolism. Our findings suggested that HA injection exhibits a greater clinical effect in cases with a high intraarticular aggrecan fragment concentration, i.e., cases in which a high amount of residual cartilage and chondrocyte metabolic activity remain. The biological markers were useful in predicting the effectiveness of HA injection for OA of the knee. (J Rheumatol 2006;33:2527–31)

Key Indexing Terms:
OSTEOARTHRITIS
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The prevalence of osteoarthritis (OA) has increased markedly with the rapid shift towards the elderly in industrially advanced countries. The major lesion of OA involves degeneration and destruction of cartilage. The early process of destruction of cartilage in OA involves degradation and conversion to lower molecular weight cartilage matrix, such as type II collagen and aggrecan, that maintains the mechanical characteristics of cartilage. Cartilage matrix is degraded and converted to low molecular components mainly by proteases produced by chondrocytes, and these destructive proteases are regulated by inflammatory cytokines and growth factors.

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Most therapeutic drugs for OA are symptom-modifying drugs that improve the clinical symptoms of OA, such as pain. Few candidate structure-modifying drugs have been confirmed clinically to inhibit cartilage destruction, and the effectiveness of these drugs is currently being investigated in a large-scale clinical study^{1,2}. One such drug therapy for OA is intraarticular injection of high molecular weight hyaluronan (HA). HA is a long linear-chain glycosaminoglycan consisting of a repetitive disaccharide unit structure that is the main component of synovial fluid. HA is important in maintaining the viscoelasticity of articular cartilage, and contributes to the maintenance of low friction, which is an important articular function, through synergistic action with cartilage. HA in synovial fluid is produced by synovial cells, and has a molecular weight of about $4\text{--}5 \times 10^6$ Da in normal subjects. In arthropathy, such as rheumatoid arthritis and OA, both the molecular weight and concentration of HA are decreased³. Such decreases in the molecular weight and concentration of HA impair lubrication between cartilage, decrease impact-absorbing ability, and cause further degeneration of the cartilage. HA receptors, including lymphocyte homing receptors, CD44, are present on the chondrocyte surface. In addition to the hydrody-

dynamic action described above, HA alters chondrocyte metabolism through receptors, and acts to protect cartilage tissue⁴. HA has been reported to exhibit cartilage-protective effects, including stimulation of chondrocyte proliferation, production of cartilage matrix, inhibition of the production of protease⁵, stimulation of the production of a protease inhibitor⁶, movement of newly synthesized aggrecan around chondrocyte⁷, and inhibition of chondrocyte apoptosis *in vitro*⁸.

HA injection improves the clinical symptoms of OA, mainly pain, in about 70% of OA cases, but nonresponders to this therapy do exist. Joint puncture is an invasive procedure that is performed with HA injection. As such, this procedure has a risk of complications, including infection. Prediction of the clinical effectiveness of this therapy enables us to perform efficient medical care.

Measurement of biological markers that are derived from components of joints, such as cartilage, synovial membrane, and bone, in synovial fluid, blood, and urine, has allowed investigation of the pathology of arthropathy⁹. Typical biological markers that reflect cartilage turnover are fragments derived from type II collagen, aggrecan, and other minor proteins that are present in cartilage. Aggrecan-derived markers are core proteins, fragments of aggrecan, glycosaminoglycans (GAG) composing aggrecan, chondroitin 6-sulfate and 4-sulfate (C6S and C4S), and keratan sulfate (KS)¹⁰⁻¹². Unlike radiograph and magnetic resonance imaging, these biological markers reflect real-time metabolism of joint components, such as cartilage and synovial membrane. Our objective was to investigate the possibility of predicting the effectiveness of HA injection from biological markers in synovial fluid collected at the initiation of intraarticular injection of HA.

MATERIALS AND METHODS

Patients. The subjects were 32 outpatients with OA of the knee (38 joints) at our orthopedic outpatient clinic who had apparent pain on movement and hydrarthrosis, and were indicated for HA injection. No concomitant steroid or nonsteroidal antiinflammatory drugs (NSAID) were administered. Patients treated with HA preparations within the past 3 months and patients with suspected rheumatoid arthritis, trauma, and suppurative arthritis were excluded. The patients' characteristics are shown in Table 1.

Intraarticular injection and collection of synovial fluid. An injection containing 25 mg of HA (molecular weight of about 900,000 Da) in 2.5 ml (super-purified hyaluronate, Seikagaku Co. Ltd., Tokyo, Japan) was injected into the knee joint once a week for one month. Synovial fluid was collected at the initiation of the first injection, and centrifuged at 3000 rpm for 15 minutes at room temperature. The supernatant was collected and immediately stored at -85°C.

Evaluation by radiography. Plain radiograms of the knee (frontal and lateral views and frontal view with weight-bearing) were acquired before the initiation of injection, and the OA stage was determined based on the Koshino classification scale¹³ (grade 0: normal, grade 1: osteosclerosis or osteophyte formation, grade 2: narrowing of the joint space to 3 mm or less, grade 3: disappearance of the joint space, grade 4: 5 mm or less bone defect of the weight-bearing surface, grade 5: 5 mm or more bone defect of the weight-bearing surface; Table 1).

Evaluation of clinical symptoms. Clinical evaluation of OA was performed, based on the criteria for judgment of therapeutic results of knee OA estab-

Table 1. Patients' characteristics. Stage of knee deformity was assessed by the radiological findings according to the Koshino criteria¹³.

	n
Sex	
Male	4
Female	34
Age, yrs	
> 60	4
60-70	5
70-80	10
< 80	19
Radiological stage	(mean = 73.4 ± 8.2)
I	11
II	5
III	13
IV	5
V	4

lished by the Japanese Orthopaedic Association (JOA score)¹⁴. In this score, pain on walking, pain on ascending and descending the stairs, range of motion, and joint swelling were rated with maximum scores of 30, 25, 35, and 10, respectively. The subjective and objective symptoms were evaluated based on the JOA scores before the initiation of intraarticular injection and at 1 month after injection. Pain was evaluated using the 100 mm visual analog scale (VAS).

Measurement of biological markers. C6S and C4S in synovial fluid were measured by high performance liquid chromatography according to the method reported by Shinmei, *et al*¹¹. Glycosaminoglycans in synovial fluid were degraded to disaccharides by treatment with chondroitinase ABC and chondroitinase AC-II, and applied to a column packed with propylamine-bound silica gel (YMC gel PA-120; YMC, Kyoto, Japan) for quantification. The aggrecan level was measured using a sandwich ELISA measurement kit (Biosource Co.) This measurement kit uses an anti-KS monoclonal antibody for capturing and a labeled anti-G1-domain monoclonal antibody as a detector, and selectively measures aggrecan molecules with HA-binding ability and a KS side chain¹⁵.

Statistical analysis. The results of measurement of the biological markers were presented as the means ± standard deviation. For analysis of the significance of between-group differences, paired t test or Wilcoxon signed-rank test was used. For analysis of correlation, Spearman's correlation coefficient by rank was used (StatView 5.0, Abacus Concepts Inc., Berkeley, CA, USA).

RESULTS

Time-course of changes in biological markers after injection. The C6S and C4S concentrations in synovial fluid showed no significant change after injection for 1 month. C6S/C4S and aggrecan concentration decreased over time, but the decrease was not significant (Figure 1).

Relationship between biological markers before injection and improvement of clinical symptoms. The relationships between the concentrations of the biological markers before HA injection and improvement of the JOA score and VAS one month after initiation of injection were investigated by regression analysis.

A significant positive correlation was observed between the C6S concentration before injection and improvement of the JOA score after one month ($r = 0.322$, $p = 0.0383$; Figure 2). No significant correlation was apparent between the C4S

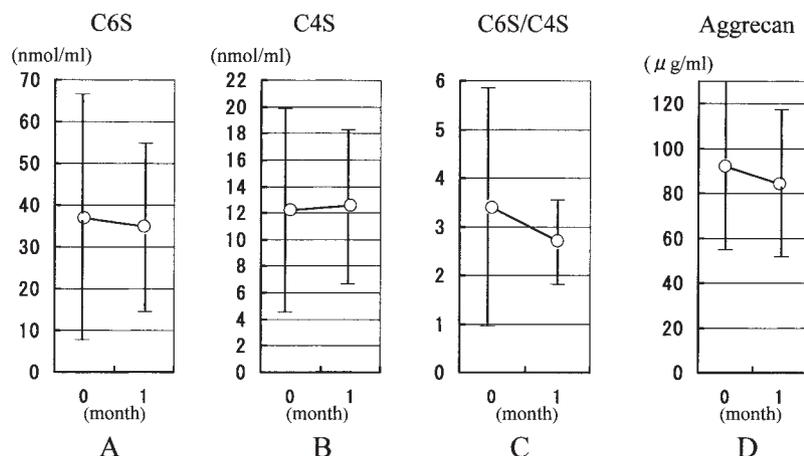


Figure 1. Time-course of changes in biological markers. HA with MW 800,000 Da was injected into the knee joint once a week for one month. C6S and C4S in synovial fluid were measured by HPLC. The aggrecan level in synovial fluid was measured using a sandwich ELISA measurement kit. Data were shown as mean \pm SD. A: C6S. B: C4S. C: C6S/C4S. D: Aggrecan.

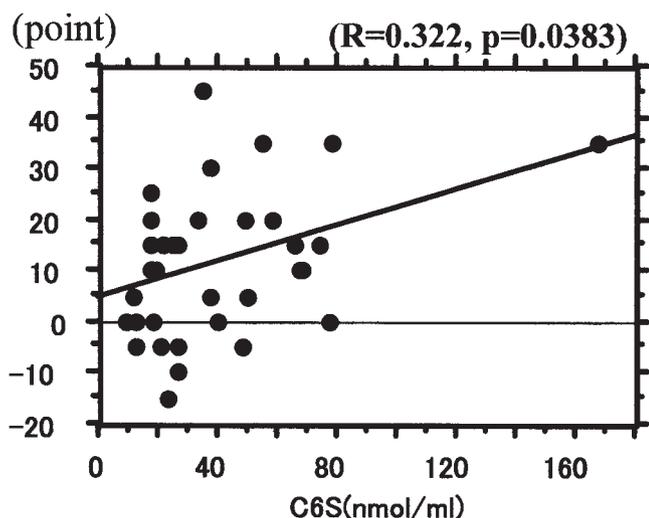


Figure 2. Relationship between C6S concentration before injection and improvement of JOA score. The relationships between C6S concentration before HA injection and improvement of the JOA score one month after initiation of injection were investigated by regression analysis.

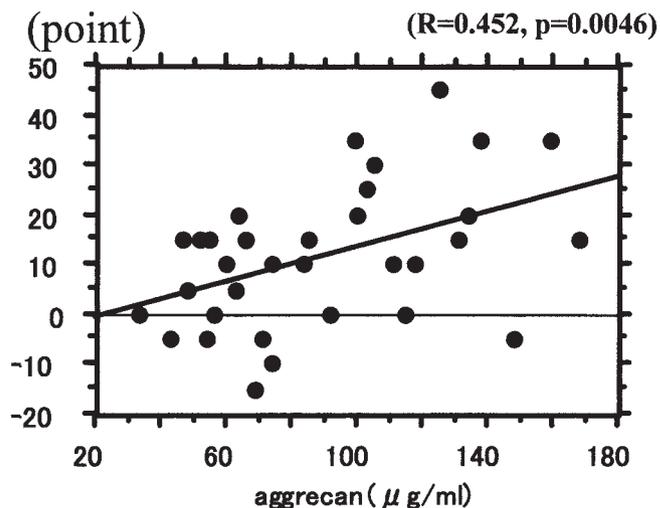


Figure 3. Relationship between aggrecan concentration before injection and improvement of JOA score. The relationships between aggrecan concentration before HA injection and improvement of the JOA score one month after initiation of injection were investigated by regression analysis.

concentration or C6S/C4S before injection and the improvement of the JOA score (data not shown). A significant correlation was observed between the aggrecan concentration before injection and improvement of the JOA score after 1 month ($r = 0.452$, $p = 0.0046$; Figure 3). In contrast, no significant correlation was apparent between the biological marker concentrations before injection and improvement of VAS (data not shown). Relationship between level of biological marker and improvement of each item of JOA score was analyzed. Significant correlation was observed between C6S concentration before injection and improvement of pain on waking ($r = 0.465$, $p = 0.0221$), and range of motion ($r = 0.433$, $p = 0.0282$) after one month. Aggrecan concentration

correlated significantly with improvement of pain on walking ($r = 0.451$, $p = 0.0268$), and pain on ascending and descending the stairs ($r = 0.577$, $p = 0.0025$) after one month. OA stage determined by radiographic findings had no significant relation with improvement of both the JOA score and VAS (Figure 4).

DISCUSSION

In OA, viscoelastic substances in synovial fluid, mainly HA, become deteriorated. Intraarticular injection of HA has been initiated based on the concept of viscosupplementation: supplementation of deteriorated HA with a novel viscoelastic substance that exhibits a hydrodynamic function¹⁶. HA has

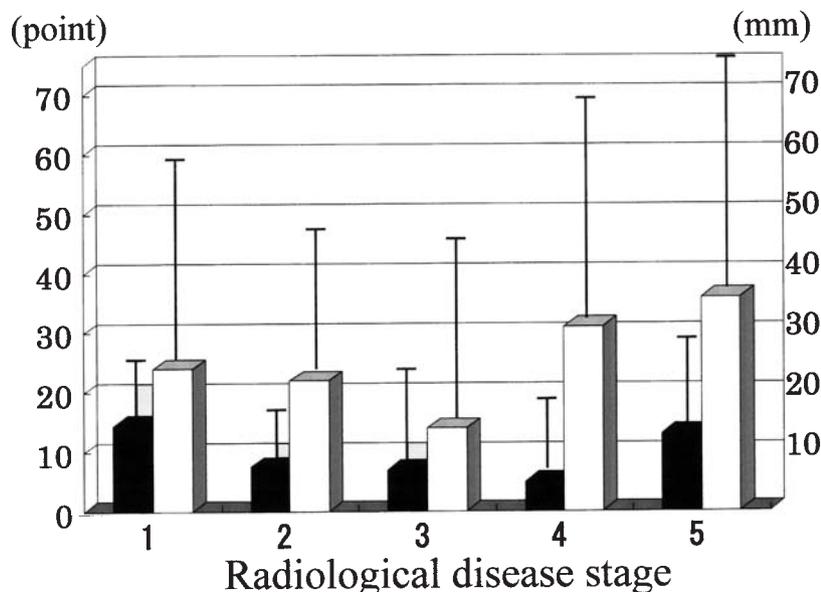


Figure 4. Radiological disease stage and improvement of JOA score and VAS. OA disease stage determined by radiographic findings had no significant relation with improvement both of the JOA score and VAS. Data were shown as mean \pm SD. Black bar: JOA score (points). White bar: VAS (mm).

been considered as a symptom-modifying drug that primarily improves the pain of OA¹⁷. HA injection apparently improved the clinical symptoms of OA, including pain, in this study.

Cartilage markers are divided into markers of synthesis and catabolism. Typical markers of catabolism reflecting the destruction of cartilage include aggrecan molecules released from aggrecan, side chain KS, and CS. There are 2 types of CS: C6S, which is abundant in healthy cartilage, and C4S, which is abundant in degenerated cartilage as with OA¹⁸. Changes in joint markers during the course of treatment of OA can help in determining the effectiveness of treatment. Yamada, *et al* measured various joint markers in synovial fluid before and after HA injection into the joint in patients with knee OA, and found that the PIICP concentration increased significantly after HA injection¹⁹. Since PIICP is a marker of the synthesis of type II collagen^{20,21}, this finding suggested that HA injection promoted matrix synthesis by chondrocytes. In studies reported by Namiki, *et al*, and Uesaka, *et al*^{22,23}, C6S and C4S levels decreased after HA injection. In our study, aggrecan level was decreased after injection of HA for one month, but this decrease was not significant. Thus, our study did not demonstrate inhibition of the degradation of cartilage aggrecan by HA injection in OA.

There has been no report of prediction of the efficacy of intraarticular injection of HA for OA based upon biological markers. We observed a significant positive correlation between the C6S concentration before injection and improvement of the JOA score after one month of injection. Furthermore, a stronger positive correlation, compared with that of C6S, was noted between the aggrecan concentration before injection and the JOA score after one month of injection.

However, radiological OA stage had no significant relation with improvement both of the JOA score and VAS. These findings suggest that improvement of the clinical symptoms after initiation of HA injection can be predicted by measurement of the fragments derived from aggrecan. Yamada, *et al* reported that the concentration of aggrecan fragments in synovial fluid decreased with the progression of OA, as a result of decreases in the amount of residual cartilage and suppression of chondrocyte metabolism with the advancement of OA²⁴⁻²⁶. In consideration of these observations, our findings indicate that HA injection is effective for cases with a high intraarticular level of aggrecan fragments. This reflects an early stage of OA in keeping with residual cartilage and chondrocyte metabolic activity.

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