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ORIGINAL ARTICLE

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Dermatan sulfate in the synovial fluid of patients with knee osteoarthritis

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Abstract Biochemical factors play an important role in osteoarthritis (OA) pathogenesis. The purpose of this study is to clarify whether the dermatan sulfate (DS) levels in the synovial fluid of patients with knee OA are related to residual cartilage. Synovial fluid was obtained from 51 OA patients. Knee radiographs were evaluated with the Kellgren–Lawrence (K/L) grading scale. The levels of the following disaccharides were measured by high-performance liquid chromatography (HPLC): DS (DSΔDi4S), chondroitin 6-sulfate (CSΔDi6S), and chondroitin 4-sulfate (CSΔDi4S). The concentration of cartilage oligomeric matrix protein (COMP) was measured by a sandwich ELISA. The levels of DSΔDi4S in Grades 0 and I OA were significantly higher than levels in Grade II ($P = 0.0458$), Grade III ($P < 0.0001$) and Grade IV ($P < 0.0001$), and we found strong relationships between the levels of DSΔDi4S and those of CSΔDi6S ($P < 0.0001$, $r = 0.705$), CSΔDi4S ($P < 0.0001$, $r = 0.750$), and COMP ($P < 0.0001$, $r = 0.699$). We conclude that the presence of DSΔDi4S reflects proteoglycan metabolism in the residual articular cartilage of OA patients. This suggests that metabolism of the small leucine-rich repeat proteoglycans decorin and biglycan, which contain chains of DSΔDi4S, is similar to that of aggrecan.

Key words Cartilage oligomeric matrix protein (COMP) · Chondroitin sulfate · Dermatan sulfate · Osteoarthritis · Synovial fluid

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Introduction

Osteoarthritis (OA) is a disabling joint disease that is characterized by the progressive destruction of articular cartilage. Diagnosis is based on clinical symptoms in combination with radiography, which is relatively insensitive, and provides only an indication of accumulated damage. Alternative methods, such as molecular markers, are therefore needed to quantitatively, reliably, and sensitively detect osteoarthritic changes in the joints at an early stage of the disease. Such molecular markers are essential for diagnosis, prognosis, and monitoring of disease progression and efficacy of therapy targeted against joint destruction.[1] The biochemical properties of articular cartilage depend on the biochemical composition and integrity of its extracellular matrix. Proteoglycans (PGs) and collagen are the major components of the extracellular matrix of articular cartilage.[2] According to the nature of their core proteins, PGs are divided into large, hyaluronan-binding PGs, like aggrecan, and small PGs, such as decorin, biglycan, epiphycan and fibromodulin.

Aggrecan is the predominant PG species in cartilage, and consists of a protein core to which glycosaminoglycan (GAG) side chains of keratan sulfate (KS) and chondroitin sulfate (CS) are attached. PG degradation products, bearing aggrecan cleavage sites, can be detected in synovial fluid by immunoassay measurement of KS and CS.[3,4] It is believed that the presence of sulfated GAG that is mainly CS, is largely a reflection of aggrecan degradation in cartilage. CS in synovial fluid has been investigated, and has attracted considerable interest as a possible marker for joint disease.[5–8] The CS can be detected by chemical analysis of its constituent disaccharides of chondroitin 6-sulfate (CSΔDi6S) and chondroitin 4-sulfate (CSΔDi4S).[9,10]

Otherwise, small PGs are components of cartilage that also have roles in maintenance of the extracellular matrix structure. A class of small PGs has also been identified, which comprises a family of structurally related, but genetically distinct molecules called the small leucine-rich repeat PGs (SLRPs). These low-weight PGs consist of a central

protein, with decorin containing one chain, and biglycan two chains, of CS or dermatan sulfate (DS) covalently linked to the N-terminal region.[11] It has been suggested that the orthogonal positioning of a small PG induces the proper spacing of the collagen fibrils by the formation of GAG chains oriented in an antiparallel manner when the core proteins are bound to adjacent fibrils.[12] Decorin and biglycan are bound to the N-terminal region of collagen VI,[13] and both SLRPs decorin and biglycan are involved in the pathophysiology of OA.[14] There was a trend in osteoarthritic cartilage toward a loss of the SLRPs from the more superficial layers of intact cartilage, where both these molecules are normally more concentrated.[15]

In this study we examined whether the chemical determination of disaccharides of DS (DSΔDi4S) provides a reliable assay for monitoring proteoglycan metabolism of osteoarthritic cartilage. Moreover, we investigated the correlation between the levels of DSΔDi4S, and those of CSΔDi6S, CSΔDi4S, and cartilage oligomeric matrix protein (COMP).

Subjects and methods

Subjects

Synovial fluid was obtained from 51 OA patients. Diagnosis of OA was based not only on history and physical examination, but also on radiographic findings, including asymmetric joint space narrowing, subchondral sclerosis, osteophyte formation, subluxation, and distribution patterns of osteoarthritic changes. Structural morphological changes on X-rays were considered the primary outcome variables for assessing the progression of OA.

Medications included nonsteroidal anti-inflammatory drugs, such as diclofenac sodium or loxoprofen sodium, which had been prescribed to reduce knee pain for all patients. Some medications in the form of creams or poultice had been applied over the skin of affected areas. However, for all patients with persistent pain despite these medications, intra-articular hyaluronan product (Artz, Supartz, manufactured by Seikagaku, Tokyo, Japan) had been injected directly into their knee joints and given as a series of some weekly joint injections. There were 51 patients in the study, including 8 males and 43 females. The mean age of the patients was 68.9 ± 11.4 years (range: 47–91 years) at study. All samples were collected with the approval of each patient.

Radiographic assessments

All patients who consented to radiographic examination of their knees were classified according to the severity of joint destruction. The evaluation of joint destruction on plain anteroposterior radiographs was performed by orthopedic surgeons.[16] The bilateral weight-bearing anteroposterior knee radiograph was taken with the patient standing with toes pointed straight ahead, knees fully extended, and weight equally distributed on both feet. The X-ray beam was aimed at the lower pole of the patella and kept parallel to the joint surface. The grading of radiographs was scored by an experienced observer, and knee radiographs were evaluated with the Kellgren–Lawrence (K/L) grading scale.[17]

Synovial fluid preparation

Synovial fluids were aspirated via a lateral infrapatellar approach, using an 18-gauge needle. Samples were collected in sterile tubes and centrifuged at 5000 × *g* for 15 min at 4°C, to remove cells and joint debris. The supernatants were stored at –80°C until subjected to biochemical assay.

Enzymatic digestion of synovial fluid and high performance liquid chromatography (HPLC) analysis

Each synovial fluid specimen was diluted 10-fold by distilled water, and a 100-µl aliquot of diluted specimen was treated with 250 mU of chondroitinase ABC (Seikagaku) plus 25 mU of chondroitinase ACII (Seikagaku) in 20 mM Tris–HCl buffer (pH 8.0). Another 100-µl aliquot was treated with 250 mU of chondroitinase ACII in 20 mM sodium acetate buffer (pH 6.0).

In both treatments, samples were incubated at 37°C for 2 h, and were ultrafiltered with Ultrafree MC (molecular size cutoff: 10 000; Millipore, Tokyo, Japan) at 15 000 rpm for 15 min. The levels of disaccharides of CSΔDi4S and CSΔDi6S, and DSΔDi4S were measured by HPLC. The ultrafiltrates were subjected to HPLC analysis according to the method of Shinmei et al.,[9] with the following modifications for the determination of each isomer: the ΔDi4S value obtained from the digest of chondroitinase ACII, which acts on CS but not on DS, was used to determine the CSΔDi4S levels, and the level of DSΔDi4S was calculated by the subtraction of the ΔDi4S value obtained from the chondroitinase ACII digestion from that obtained from the chondroitinase ABC plus chondroitinase ACII digestion, which can cleave both CS and DS.

Quantification of serum COMP

COMP was measured by a sandwich ELISA using two monoclonal antibodies directed against separate antigenic determinants on the human COMP molecule (AnaMar Medical, Lund, Sweden).

Statistical analysis

Data were analyzed using SAS StatView for Windows statistics package. For correlation analysis, we used the Spearman rank correlation test. The Mann–Whitney *U*-test was used to compare the parameters of the various patient groups. *P* values less than 0.05 were considered statistically significant.

Table 1. Synovial fluid components from osteoarthritic knee joints according to the Kellgren–Lawrence (K/L) grading scale

	K/L Grading scale			
	0, I (n = 10)	II (n = 9)	III (n = 24)	IV (n = 8)
DSΔDi4S (nmol/ml)	3.43 ± 2.07	1.39 ± 0.85*	0.74 ± 0.55**	0.74 ± 0.78**
COMP (U/l)	128.5 ± 43.5	84.9 ± 28.0**	79.9 ± 44.1**	53.9 ± 0.165**
CSΔDi6S (nmol/ml)	298.1 ± 122.3	118.9 ± 41.5**	57.0 ± 16.0**	54.9 ± 24.4**
CSΔDi4S (nmol/ml)	36.5 ± 8.6	22.9 ± 4.3**	16.6 ± 3.1**	15.3 ± 4.9**
4S + 6S (nmol/ml)	334.6 ± 130.5	141.8 ± 45.2**	73.5 ± 18.8**	70.2 ± 28.6**
6S/4S	7.93 ± 1.52	5.10 ± 0.97**	3.41 ± 0.53**	3.47 ± 0.70**

Levels at K/L grading scale 0 and I compared to II through IV: *P < 0.05, **P < 0.01

Results

Relationship between severity of joint destruction and DSΔDi4S levels in OA synovial fluid

We analyzed the levels of DSΔDi4S in synovial fluid by HPLC, and compared them in relation to radiological stages of OA. The levels of samples from Grade 0 and I (n = 10) were 3.43 ± 2.07 nmol/ml (mean ± SD); those from Grade II (n = 9) were 1.39 ± 0.85 nmol/ml; those from Grade III (n = 24) were 0.74 ± 0.55 nmol/ml; and those from Grade IV (n = 8) were 0.74 ± 0.78 nmol/ml (Table 1). These results showed that the levels of DSΔDi4S in Grade 0 and I were significantly higher than those of Grade II (P = 0.0458), Grade III (P < 0.0001), and Grade IV (P < 0.0001), and levels in Grade II were higher than those in Grade III and Grade IV. The levels of these molecules in Grades 0 and I were significantly higher than those in Grades II–IV. There were similar correlations seen for CSΔDi4S, CSΔDi6S, and COMP. According to K/L grading scale the tendency of decreasing of DSΔDi4S was similar in the pattern of CSΔDi4S, CSΔDi6S, and COMP.

Correlation of PG levels in synovial fluid

We then analyzed the correlation between the levels of DSΔDi4S and the levels of CSΔDi6S, CSΔDi4S, and COMP. The levels of DSΔDi4S significantly correlated with those of CSΔDi6S (P < 0.0001, r = 0.705) (Fig. 1). The levels of DSΔDi4S also correlated strongly with CSΔDi4S levels (P < 0.0001, r = 0.705) (Fig. 2), and with COMP levels (P < 0.0001, r = 0.699) (Fig. 3).

Discussion

Recent glycobiology studies have suggested that there are fundamental biological functions for CS and DS, which are both widely distributed as GAG side chains of proteoglycans in the extracellular matrix and at cell surfaces.[9,11] However, there are few reports concerning the role of DS in the pathophysiology of OA.[11] Until this study, DSΔDi4S levels had never been measured in the serum and joint fluid of patients with OA. We first tried to measure the levels of

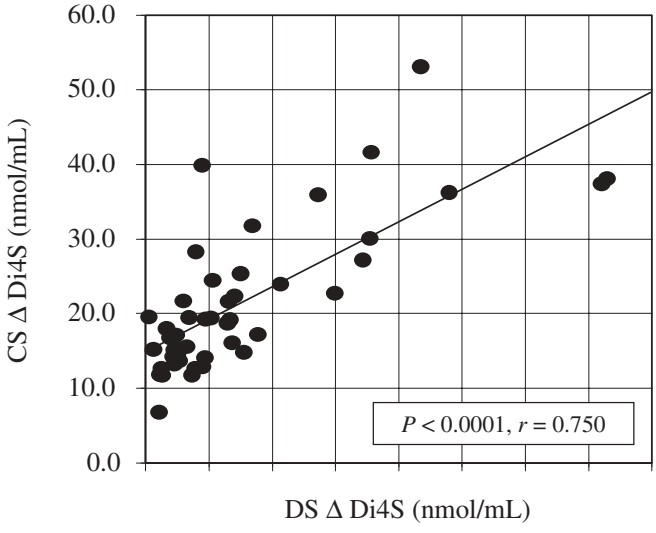

Fig. 1. Correlations between DSΔDi4S and CSΔDi4S in synovial fluid from patients with osteoarthritis (OA). The levels of DSΔDi4S correlated with those of CSΔDi4S (P < 0.0001, r = 0.750)

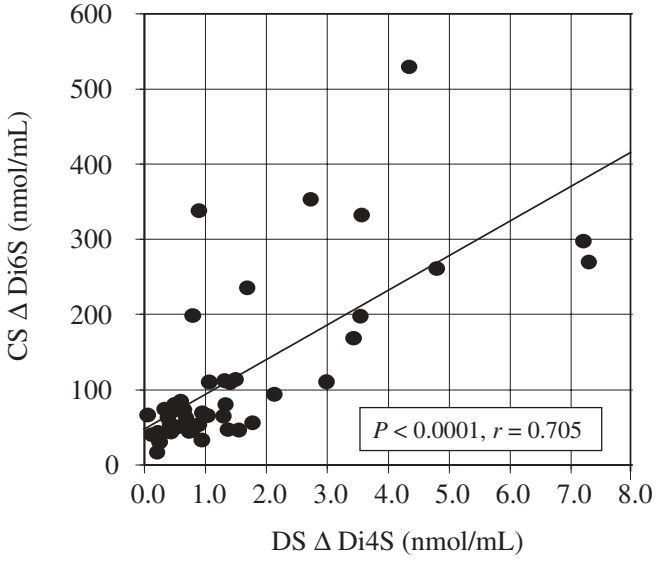

Fig. 2. Correlations between DSΔDi4S and CSΔDi6S in synovial fluid from patients with OA. The levels of DSΔDi4S correlated strongly with those of CSΔDi6S (P < 0.0001, r = 0.705)

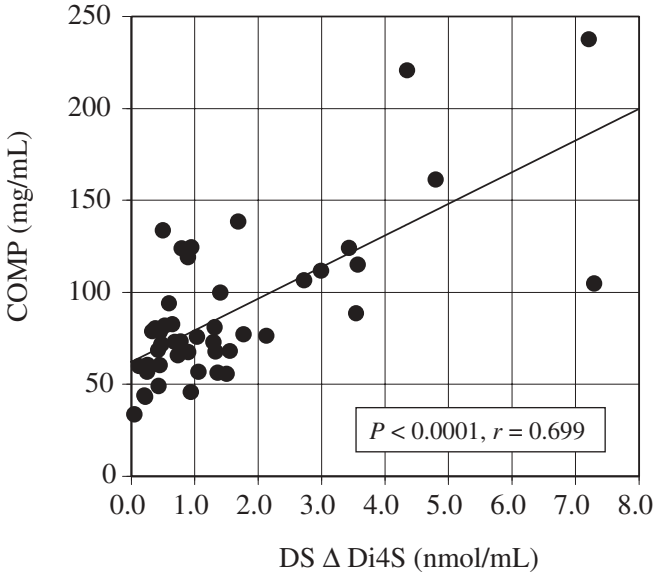

Fig. 3. Correlations between DSΔDi4S and COMP in synovial fluid from patients with OA. The levels of DSΔDi4S also correlated well with those of COMP ($P < 0.0001$, $r = 0.699$)

DSΔDi4S in serum but, unexpectedly, we were not able to detect DSΔDi4S. Therefore, in this study we decided to measure the levels of DSΔDi4S in the synovial fluid of patients with OA, and compare the results with measured levels of CSΔDi6S, CSΔDi4S, and COMP. The levels in synovial fluid of CS isomers such as CSΔDi6S and CSΔDi4S reflect the PG metabolism of joint tissues; hence, PG levels may be useful in the diagnosis of joint diseases, as well as for the prediction of the degree of resulting articular cartilage destruction.[9]

Until this study, we speculated that the metabolism of DSΔDi4S in articular cartilage might be different from the metabolism of CSΔDi6S and CSΔDi4S. Aggrecan is the predominant PG in cartilage, and small PGs are also cartilage components having the function of maintaining the structure of the extracellular matrix. Bock et al. reported that the levels of transcription and translation for decorin and biglycan were up-regulated in the late stages of OA, probably in an effort to compensate for the general PG loss seen in advanced OA.[14] However, our results show that the levels of DSΔDi4S are significantly correlated with those of CSΔDi6S and CSΔDi4S in the synovial fluid of patients with OA. Moreover, significantly higher amounts of DSΔDi4S were observed in synovial fluid samples from patients classified as Grades 0 and I, and the levels from samples in Grades II, III, and IV gradually decreased. This correlation was the same for the levels of CSΔDi6S and CSΔDi4S. There have been a few reports that the levels of CSΔDi6S and CSΔDi4S in osteoarthritic synovial fluid correspond to the degree of the residual articular cartilage,[9,10] and the levels in those reports were similar to our results.

These findings suggest that the levels of DSΔDi4S in synovial fluid reflect PG metabolism of the residual articular cartilage in patients with OA. The results of this study indicate that the metabolism of SLRPs such as decorin and biglycan is similar to the metabolism of aggrecan, which is the dominant PG in cartilage. However, the PGs of CSΔDi4S, CSΔDi6S, and DSΔDi4S were also detected throughout the ligaments and menisci.[18–20] Further study is necessary and desirable to investigate the relationships between DS and the degradation of the ligaments and menisci.

COMP is a member of the thrombospondin family. It is a noncollagenous extracellular matrix protein found predominantly in cartilage, but is also present in tendons, ligaments, and menisci.[21] COMP is a calcium-binding protein of high molecular weight (>500 kDa), and is composed of five identical subunits.[22,23] The carboxy terminal globular domain of native COMP binds to collagens I, II, and IX.[24,25] These characteristics suggest that COMP may be involved in regulating fibril formation and maintaining the integrity of the collagen network.[26] As a result, COMP has been reported to be one of the most useful markers for joint diseases.[27] The first assay for COMP measurement in serum and synovial fluid was described in 1992, and was based on a polyclonal antiserum and performed as an inhibition ELISA.[28]

In this study, we found that the levels of DSΔDi4S were significantly correlated not only with the levels of CSΔDi6S and CSΔDi4S, but also with that of COMP. Moreover, higher amounts of COMP were observed in samples from patients classified as Grades 0 and I, and, similar to DSΔDi4S, the levels gradually decreased in order in samples from Grades II, III, and IV. These findings suggest that the metabolism of DS is similar to the metabolism of COMP, which is produced mainly from cartilage, but is also detected throughout tendons, ligaments, and menisci.

This is the first report showing that the levels of DSΔDi4S in synovial fluid reflect residual cartilage in patients with OA. We conclude the following from our data: (1) the levels of DSΔDi4S in synovial fluid reflect small leucine-rich repeat PG metabolism of the residual articular cartilage in patients with OA, and, the metabolism SLRPs such as decorin and biglycan is parallel to that of aggrecan in osteoarthritic cartilage; (2) the levels in synovial fluid from patients with OA, of DSΔDi4S, as well as CSΔDi4S, CSΔDi6S, and COMP, are reliable markers reflecting the destruction of articular cartilage; (3) CSΔDi4S, CSΔDi6S, COMP, and DSΔDi4S were also detected throughout the tendons, ligaments, and menisci. Therefore, further study is necessary to investigate the relationships between DS and the degradation of the ligaments and menisci.

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