Chondronectin in human synovial fluid

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SUMMARY Chondronectin is a cartilage matrix protein that specifically mediates the attachment of chondrocytes to type II collagen. A monoclonal antibody to chondronectin was used in a competitive enzyme linked immunosorbent assay (ELISA) and Western blot assay to detect, measure, and characterise chondronectin in human synovial fluid. Chondronectin was detected in all 18 rheumatoid and 11 osteoarthritic synovial fluids examined. Chondronectin concentrations in synovial fluid were lower than those in plasma and correlated most strongly with concentrations of synovial fluid albumin and fibrinogen. These results suggest that synovial fluid chondronectin is in part derived from plasma. The role of chondronectin as a marker of cartilage destruction and potential mediator of pannus formation is discussed.

Key word: cartilage.

Chondronectin is an extracellular matrix protein which specifically mediates the attachment of chondrocytes to type II collagen.¹ Chondronectin has been localised to human cartilage and vitreous and to date has not been found in other tissues. It is a 180 000 molecular weight protein composed of disulphide linked 75 kilodalton subunits. Like fibronectin, chondronectin circulates in plasma, though at concentrations approximately one tenth of circulating fibronectin (~10 mg/l).

Several matrix proteins have been detected in the synovial fluid of patients with rheumatic disease. It has been suggested that the presence of matrix proteins and their degradation products may be sensitive indicators of matrix breakdown and repair in arthritis. Synovial fluid fibronectin concentrations are higher than plasma fibronectin levels in rheumatoid arthritis (RA), correlate with synovial fluid white blood cell counts, and appear to be an indicator of proliferative disease.² Collagen I and III peptides have also been detected in inflammatory synovial fluid. The quantity of collagen peptide recovered in synovial fluid pellets has been shown to correlate with the degree of joint destruction radiographically.³

Although chondronectin has been localised to cartilage matrix, it is not known if it is present in the

synovial fluid of patients with rheumatic disease. To determine whether chondronectin is present in human synovial fluid and if it may be useful as a marker of cartilage matrix destruction we used a monoclonal antibody to human chondronectin to detect and measure chondronectin in osteoarthritic and rheumatoid synovial fluid. Relations between synovial chondronectin concentrations, plasma chondronectin concentrations, and concentrations of other synovial fluid proteins, including fibronectin, are described.

Patients and methods

PATIENTS

Synovial fluid was obtained from rheumatoid and osteoarthritic patients undergoing diagnostic or therapeutic arthrocentesis. Patients with RA had definite or classical disease; patients with osteoarthritis (OA) had typical clinical and radiographic findings. Synovial fluid was collected aseptically, centrifuged at 1000 g for 15 minutes to remove cellular elements, and the supernatant was frozen at –30°C until use.

ANTICHONDRONECTIN

A monoclonal antibody to human chondronectin was prepared as previously described.⁴

COMPETITIVE ELISA FOR CHONDRONECTIN

Hyaluronidase treated synovial fluid (250 µl) was

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incubated with 250 μ l of monoclonal antichondronectin ascites (1:1400) in 12 $\times$ 75 polystyrene tubes for 16 hours at 22°C. These antigen-antibody mixtures (200 μ l) were then transferred to 96 well microtitre plates (Sarsted) that had been previously treated with chondronectin antigen (4 μ g/well) in 0.05 M Na₂CO₃, 0.02% azide, pH 9.6, for 16 hours at 4°C. After incubation for one hour at 37°C the antigen-antibody mixtures were aspirated and the wells were washed three times with phosphate buffered saline—0.05% Triton X-100. Goat anti-mouse polyvalent antibody conjugated to alkaline phosphatase (1:200) was then added for one hour at 37°C. After washing phosphatase substrate (*p*-nitrophenyl phosphate) was added and colour development was monitored at 405 nm with a Titertek ELISA plate reader. Unknown antigen concentration, inversely proportional to optical density, was calculated from a standard curve constructed from dilutions of partially purified chondronectin of known concentration. A standard curve was included on each plate and for each set of unknowns. A pooled normal human plasma was calculated to have a chondronectin concentration of 10.1 mg/l. Variation of the plasma chondronectin ELISA assay is 10% (SEM) (V Horn, H Varner, H Kleinman, in preparation).

MEASUREMENT OF OTHER SYNOVIAL FLUID PROTEINS

Albumin and fibrinogen were measured by radial immunodiffusion using commercially available plates (Calbiochem). Fibronectin was measured by electro-immunodiffusion as previously described.²

GEL ELECTROPHORESIS

The size distribution of chondronectin antigen in synovial fluid was evaluated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), performed by the method of Laemmli⁵ followed by Western blotting.⁶ Nitrocellulose papers were blocked with 3% bovine serum albumin-trometamol (TRIS) buffered saline and then incubated with antichondronectin ascites (1:50) for one hour at 22°C. After washing the paper was incubated with goat antimouse IgG coupled to peroxidase (1:500) for one hour at 37°C followed by peroxidase substrate.

STATISTICS

Statistical significance was determined using Student's *t* test for paired or unpaired samples. Regression statistics for multiple variables were determined using the Spearman rank correlation test (Corr program—Crunch software).

Results

DETECTION OF CHONDRONECTIN IN HUMAN SYNOVIAL FLUID

Chondronectin was detected by ELISA in all 29 synovial fluids studied. Comparison of the dilution-binding curves in synovial fluid and normal plasma showed that they were essentially parallel and similar in shape. When chondronectin in synovial fluid was examined by SDS-PAGE and immunoblotting under reducing conditions an intact subunit of approximately 75 kilodaltons was observed in both RA and OA synovial fluid (Fig. 1).

MEASUREMENT OF CHONDRONECTIN IN SYNOVIAL FLUID (Table 1)

Chondronectin concentrations in 29 synovial fluid samples ranged from 0.9 to 10.6 mg/l with a mean

Fig. 1 Western blot of 8% SDS-PAGE gel under reducing conditions. Lane 1=purified chondronectin (10 μ g); lane 2=RA synovial fluid (No 13, Table 1) 90 μ l, containing 3.2 μ g chondronectin; lane 3=OA synovial fluid (No 23, Table 1) 90 μ l, containing 2.9 μ g chondronectin. Proteins transferred to nitrocellulose were incubated with antichondronectin monoclonal antibody (1:50) and then peroxidase conjugated goat antimouse immunoglobulin.

Table 1 Chondronectin concentrations in human rheumatoid and osteoarthritic synovial fluid

Sample No	Chondronectin (mg/l)	Fibronectin (mg/l)	Albumin (g/l)	Fibrinogen (g/l)	Diagnosis
1	3.96	277	23	3.6	RA
2	6.86	429	30	5.1	RA
3	7.30	536	17	4.3	RA
4	9.24	610	23	7.0	RA
5	8.62	520	23	6.0	RA
6	9.68	748	23	9.4	RA
7	9.50	763	17	8.1	RA
8	7.66	520	37	15.3	RA
9	8.80	530	23	5.5	RA
10	8.80	1290	51	10.4	RA
11	8.80	365	23	5.5	RA
12	7.30	184	1	1.0	RA
13	10.56	250	51	9.0	RA
14	6.27	888	7	0	RA
15	4.14	880	11	1.5	RA
16	8.45	705	16	15.0	RA
17	10.23	780	19	4.0	RA
18	10.20	495	31	9.0	RA
19	7.39	60	41	4.5	OA
20	9.24	320	23	6.5	OA
21	0.88	242	15	3.7	OA
22	8.45	765	41	7.7	OA
23	9.33	96	32	4.5	OA
24	5.28	376	29	5.5	OA
25	9.77	521	48	4.5	OA
26	5.28	80	39	5.5	OA
27	7.30	132	20	3.7	OA
28	6.78	448	20	11.9	OA
29	9.42	911	13	5.5	OA
Mean (SD)	7.8 (2.2)	508 (295)	26 (13)	6.3 (3.6)	

(SD) of 7.8 (2.2) mg/l. Chondronectin concentrations were similar in RA and OA synovial fluid. In 18 RA fluids the mean (SD) chondronectin concentration was 8.1 (3.2) mg/l, while in 11 OA fluids the mean (SD) chondronectin concentration was 7.2 (2.6) mg/l. The chondronectin concentration of a pooled normal human standard plasma was 10.1 mg/l.

When chondronectin was measured in four simultaneously obtained pairs of synovial fluid and plasma the plasma concentration exceeded that in the synovial fluid in each case (Table 2). The mean

plasma chondronectin concentration (13.4 mg/l) significantly exceeded the mean synovial fluid chondronectin concentration (7.9 mg/l).

RELATIONS BETWEEN SYNOVIAL FLUID CHONDRONECTIN, FIBRONECTIN, ALBUMIN, AND FIBRINOGEN CONCENTRATIONS

When all RA synovial fluids were examined the chondronectin concentrations correlated positively with concentrations of fibrinogen ($r=0.35$, $p=0.03$) and albumin ($r=0.30$, $p=0.06$). Chondronectin concentrations did not correlate strongly with fibronectin concentrations ($r=0.24$, $p=0.1$).

Table 2 Chondronectin concentrations in paired synovial fluid and plasma samples

Sample	Disease	Chondronectin (mg/l) in:	
		Synovial fluid	Plasma
Top	RA	11.1	15.3
Arc	RA	9.4	13.4
Lew	OA	0.9	9.7
Nar	Gout	10.2	15.3
Mean		7.9	13.4

Discussion

We have demonstrated the presence of immuno-reactive chondronectin in synovial fluid from patients with RA and OA. The mean synovial fluid chondronectin concentration (7.8 mg/l) was approximately 80% of that measured in pooled normal human plasma. Plasma chondronectin has not been systematically studied in patients with arthritis, but

preliminary data suggest that chondronectin concentrations in the plasma of patients with arthritis are similar or slightly higher than normal plasma concentrations. The measurement of chondronectin in synovial fluid by competitive ELISA did not appear to be influenced by synovial fluid as dilution-binding curves were parallel and nearly identical whether performed in the presence of synovial fluid or plasma. Synovial fluid concentrations also appeared not to reflect the presence of chondronectin proteolytic degradation products in the inflamed joint as immunoblotting studies showed the presence of an intact 75 kilodalton subunit in synovial fluid as well as plasma. In addition, lower molecular weight bands corresponding to potential proteolytic fragments were not seen.

Although chondronectin concentrations in RA fluids were slightly higher than those in OA fluids, these differences were not statistically significant. The ratio of chondronectin to fibronectin was approximately 1.7-fold higher in OA fluid than in RA fluid. This may primarily represent differences in fibronectin concentrations. Alterations in relative quantities of matrix proteins in synovial fluid may influence the phenotypic behaviour of synovial cells. Further studies will be necessary to determine the use of matrix protein measurement in the clinical and pathological evaluation of joint destruction.

When the subgroup of RA fluids was analysed the synovial fluid chondronectin concentrations were found to correlate positively with concentrations of synovial fluid fibrinogen and albumin. Synovial fluid chondronectin correlated less strongly with concentrations of fibronectin, which appears to be locally synthesised. Albumin and fibrinogen are not locally synthesised and enter synovial fluid by diffusion from plasma in the presence of inflammation.⁷ Thus albumin and fibrinogen concentrations in synovial fluid never exceed those in plasma, though their concentration in synovial fluid appears to correlate with the degree of inflammation present. These data taken together with the data on plasma-synovial

fluid pairs (Table 2) suggest that synovial fluid chondronectin is in part derived from plasma. Alternatively, chondronectin released from cartilage matrix may enter the synovial space in larger quantities but may deposit in an insoluble form, inaccessible to measurement in synovial fluid.

The function of chondronectin in synovial fluid is not known. In RA chondrocytes released from partially degraded RA cartilage matrix may contribute to the formation of pannus.⁸ The presence of soluble chondronectin in synovial fluid may mediate the recruitment of chondrocytes to the synovial space, where under the influence of fibronectin they could undergo alteration to a phenotype characteristic of cells derived from pannus.

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