Glycosaminoglycans in patellar cartilage

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We obtained samples of patellar cartilage at the time of surgery from 20 patients: 11 with normal and 9 with macroscopic cartilaginous degeneration. The glycosaminoglycans were separated by cellulose acetate electrophoresis, and the relative content of major glycosaminoglycans was measured by optic scanning. The total content of glycosaminoglycans was estimated by uronic acid analysis. We found that degenerated cartilage contained an increase in dermatan sulfate and hyaluronic acid and a decrease in chondroitin 6-sulfate and total uronic acid concentration, with only slight changes in chondroitin 4-sulfate and keratan sulfate compared with normal cartilage.

The stiffness of cartilage is directly correlated with the proteoglycans and their content of glycosaminoglycans (Kempson et al. 1970). Proteoglycans exert a swelling pressure that is constantly restrained by the collagen network in which they are entrapped. Although glycosaminoglycans from normal and arthrotic cartilage from many joints have been analyzed, surpprisingly little is known about their compositions in patellar cartilage. Degeneration of the patellar cartilage is common and occurs early. We studied glycosaminoglycans of degenerated and normal patellar cartilage.

Patients and methods

Samples of patellar cartilage were obtained at the time of surgery from 20 patients: 11 with normal and 9 with macroscopic degeneration of the cartilage (Table 1). All the subjects gave informed consent to participate in the study. The biopsies (5–10 mg) were taken from the middle of the medial patellar facet or from the degenerated area and cut at a thickness of 300–500 µm parallel to the articular surface (Jones et al. 1977).

The biopsies were defatted and dried in three changes of acetone, acetone/ether (1:1), and ether and kept in a desiccator at room temperature until constant weight was attained. The defatted, dried tissue biopsy was cut into small pieces, and aliquots of 5–10 mg were digested with pronase (0.5 mg/mg dried tissue in 2.0 ml distilled water at 46 °C for 24 h). Five volumes of ethanol saturated with NaCl were added and left overnight at 4 °C. The precipitate was collected by centrifugation at 1720 *g* for 20 min at 4 °C, dried, and redissolved in 2.5 ml sodium acetate buffer (0.05 M, pH 5.0). The redissolved mixture was centrifuged at 1720 *g* for 20 min at 20 °C. The supernatant was decanted in a tube, and sodium acetate buffer (0.05 M, pH 5.0) was again added to the precipitate, which was centrifuged at 1720 *g* for 20 min at 20 °C. The supernatant was transferred to the tube with the first supernatant, and 200 µL of a 5 percent centyltrimethyl ammonium bromide solution was added and left overnight at room temperature. The precipitated glycosaminoglycan-centyltrimethyl ammonium bromide complex was collected by centrifugation at 1720 *g* for 20 min at 4 °C and washed three times with ethanol saturated with NaCl to remove centyltrimethyl ammonium bromide. The purified sodium-glycosaminoglycan was dried and redissolved in 100 µL 0.1 M NaOH followed by 900 µL distilled water for further analysis. Uronic acid analysis was performed by the procedure described by Blumenkrantz and Asboe-Hansen (1973) using glucuronic acid as the standard.

Cellulose acetate electrophoresis was a modification of the method described by Capelletti et al. (1979). Cellulose acetate electrophoresis plates Titan III (2.5 x 7.6 cm) were purchased from Helena Laboratories (Beaumont, TX, USA). The electrophoresis apparatus

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(Model SAE 2761) with a water cooling system was obtained from Shandon Scientific Company (City, England). The temperature of the cooling water in the refrigerating disk was 8 °C during migration. The buffer solution used in the electrode compartment was barium acetate 0.2 M, pH 4.

Samples of the isolated glycosaminoglycans containing 1–2 nmol uronic acid were run at 250 V in a 0.2 M barium acetate buffer, pH 4, for 15 min. A second run of 15 min in 0.2 M barium acetate, pH 4, containing 15 percent ethanol was followed by a third run of 20 min in 0.2 M barium acetate, pH 4, containing 30 percent ethanol; and finally a further 50 min monodimensional run in 0.2 M barium acetate, pH 4, containing 50 percent ethanol, resulted in the separation of hyaluronic acid, dermatan sulfate, keratan sulfate, chondroitin 4-sulfate, and chondroitin 6-sulfate. After electrophoresis, the cellulose acetate plates were stained with 0.1 Alcian Blue in 5 percent acetic acid containing 10 percent ethanol, and then were destained by changes of 7.5 percent acetic acid.

Identity of the bands in both standard and experimental samples was confirmed using specific degradation by chondroitin ABC-lyase and testicular hyaluronidase. Quantification of the bands was accomplished by densitometric scanning of the Alcian Blue-stained cellulose acetate strips using a Vitatron TLD 100 densitometer with a 577-nm filter and automated integration of the curves. The integrated areas were measured in arbitrary units. Linearity between the integrated area in units and the concentration of the individual glycosaminoglycans was confirmed with standards. The relative content of the individual glycosaminoglycans in the biopsy samples was calculated as the area beneath the curves in percentage of the total area. The concentration of the total glycosaminoglycans in the biopsy samples was estimated as the uronic acid concentration.

The Wilcoxon rank sum test for unpaired data was used.

Results

A decrease ($P < 0.01$) in uronic acid concentration was found in samples from patients with degenerated cartilage when compared with samples from patients with normal cartilage (Table 1). The relative distribution of the individual glycosaminoglycans was measured in each biopsy. It was possible to identify glycosaminoglycans in all the tissue samples. In the degenerated cartilage, as compared with normal cartilage, there was an increase ($P < 0.05$) in the dermatan sulfate and hyaluronic acid fractions, and a decrease ($P < 0.05$) in the chondroitin 6-sulfate fraction, with only slight changes (NS) in the chondroitin 4-sulfate and keratan sulfate fraction (Table 2).

Table 1. Uronic acid (mean, *SEM*) in patellar cartilage. Patients 1–9, degenerated cartilage. Patients 10–20, normal cartilage. Uronic acid nmol/mg dried, defatted tissue

	Age, sex, duration (mo)			Degeneration area	Torn meniscus	Uronic acid (nmol/mg)
1	45	M	4	M	–	17.9
2	22	M	24	M	–	21.4
3	48	F	2	M	–	33.4
4	58	F	6	M	–	29.2
5	41	F	36	M	L	33.6
6	54	M	14	M	M	20.5
7	35	F	3	C	–	37.3
8	34	M	60	M/C	–	47.1
9	22	F	2	M/C	–	18.1
						28.7 *3.6*
10	24	M	1	–	M	82.2
11	41	F	3	–	L	83.8
12	25	M	24	–	M	123
13	36	M	6	–	M	50.7
14	49	M	6	–	–	67.5
15	31	F	72	–	–	104
16	24	F	12	–	–	79.6
17	48	F	7	–	–	75.9
18	12	M	36	–	–	123
19	40	F	6	–	–	50.9
20	16	F	36	–	–	70.8
						82.8 *7.5* $P < 0.01$

C Central L Lateral M Medial

Table 2. The dermatan sulfate (DS), hyaluronic acid (HA), chondroitin 4-sulfate (CH-4-S), chondroitin 6-sulfate (CH-6-S), and keratan sulfate (KS) fractions as percentages of the total glycosaminoglycan (GAG) content. Mean, *SEM*

	DS	HA	Ch-4-S	Ch-6-S	KS
Degenerate cartilage	13.1 *1.5*	22.0 *2.9*	10.1 *1.3*	34.9 *4.2*	20.3 *3.5*
Normal cartilage	9.1 *1.6*	14.7 *1.0*	9.7 *1.3*	48.2 *1.8*	18.1 *1.9*
P-values	< 0.05	< 0.05	NS	< 0.05	NS

Discussion

A decreased level of total glycosaminoglycans in arthrosis has been observed by others (Collins 1948, Mankin and Lipiello 1971, Sweet et al. 1977); and also the presence of dermatan sulfate, hyaluronic acid, keratan sulfate, chondroitin 4- and 6-sulfate in integumentary cartilage has been reported earlier (Yusipova and Kriuk 1979, Rosenberg et al. 1985).

The uronic acid content was 35 percent lower in degenerated patellar cartilage than in macroscopic normal cartilage, indicating a sharp drop in the sulfated polysaccharide content of this tissue, which accords with reports by Hirsch (1944) and Zanoli et al. (1981). According to our investigations the changes concerned mainly dermatan sulfate, hyaluronic acid, and chondroitin 6-sulfate; and the decrease in the total glycosaminoglycan content was related to the reduced content of chondroitin 6-sulfate.

In immature cartilage and early arthrosis, the keratan sulfate content is relatively low (Muir 1977). In late stages of arthrosis the proportion of keratan sulfate to chondroitin 6-sulfate increases (Thompson et al. 1979). In degenerated patellar cartilage the proportion of keratan sulfate to chondroitin 6-sulfate resembled late stages of arthrosis.

Degeneration of the patellar cartilage in early cases is confined to the central and medial parts of the patella. The reason for this location is not known. Wiberg (1941) found that the medial patellar facet only touched the medial femoral condyle with a small portion of its surface; but at the beginning of flexion, this contact increases outwardly, and beyond 90° the point of contact between the medial facet and the medial femoral condyle was the same as it is at incipient degeneration of the patellar cartilage. Wiberg´s report has been used to support the theory that too much pressure on the small remaining area of contact on the medial side is the cause of the degeneration, but this has also been used to support the suggestion that it is caused by too little pressure on the rest of the facet (Wiles et al. 1956). Hirsch (1944) showed in healthy and degenerated patellar cartilage that whenever the content of chondroitin sulfate was reduced the cartilage exhibited impaired resistance to loading.

References

Blumenkrantz N, Asboe-Hansen G. New method for quantitative determination of uronic acids. Anal Biochem 1973;54(2):484–9.

Cappelletti R, Del Rosso M, Chiarugi V P. A new electrophoretic method for the complete separation of all known animal glycosaminoglycans in a monodimensional run. Anal Biochem 1979;99(2):311–5.

Collins D H. The pathology of articular and spinal diseases. Edward Arnold & Co, London 1949:74–115.

Hirsch C. The pathogenesis of chondromalacia of the patella. A physical, histologic and chemical study. Acta Chir Scand 1944;(Suppl 83).

Jones I L, Larsson S E, Lemperg R. The glycosaminoglycans of human articular cartilage: concentration and distribution in different layers in the adult individual. Clin Orthop 1977;(127):257–64.

Kempson G E, Muir H, Swanson S A, Freeman M A. Correlations between stiffness and the chemical constituents of cartilage on the human femoral head. Biochim Biophys Acta 1970;215(1):70–7.

Mankin H J, Lippiello L. The glycosaminoglycans of normal and arthritic cartilage. J Clin Invest 1971;50(8):1712–9.

Muir H. Molecular approach to the understanding of osteoarthrosis. (Heberden Oration, 1976). Ann Rheum Dis 1977;36(3):199–208.

Rosenberg L C, Choi H U, Tang L H, Johnson T L, Pal S, Webber C, Reiner A, Poole A R. Isolation of dermatan sulfate proteoglycans from mature bovine articular cartilages. J Biol Chem 1985;260(10):6304–13.

Sweet M B, Thonar E J, Immelman A R, Solomon L. Biochemical changes in progressive osteoarthrosis. Ann Rheum Dis 1977;36(5):387–98.

Thompson R C Jr, Oegema T R Jr. Metabolic activity of articular cartilage in osteoarthritis. An in vitro study. J Bone Joint Surg (Am) 1979;61(3):407–16.

Wiberg G. Roentgenographic and anatomic studies on the femoropatellar joint. Acta Orthop Scand 1941;12:319–23.

Wiles P, Andrews P S, Devas M B. Chondromalacia of the patella. J Bone Joint Surg (Br) 1956;38:95–9.

Yusipova N A, Kriuk A S. Articular cartilage, blood serum glycosaminoglycans and glycoproteins in osteoarthritis deformans. Clin Chim Acta 1979;94(1):9–21.

Zanoli S, Raffaelli P, Rindi S, De Luca G. Biochemical changes in the articular cartilage of the patella and femoral condyles in the lateral hyperpatellar syndrome. Ital J Orthop Traumatol 1981;7(2):215–22.