Glycosaminoglycans in normal and osteoarthrotic human temporomandibular joint disks

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Glycosaminoglycans in normal and osteoarthrotic temporomandibular joint disks were studied by means of high-performance liquid chromatography methods. Normal disk tissue contains galactosaminoglycans (chondroitin sulfate and dermatan sulfate) as the main polysaccharides and with smaller amounts of hyaluronate and heparan sulfate. The galactosaminoglycans are mainly sulfated in 6-position, and some of the disaccharides contain iduronic acid. There was a slight general variation in glycosaminoglycan concentration with increasing age. In the severely arthrotic disks the content of glycosaminoglycans was considerably lower than in normal disk tissue. This decrease was far more extensive than that observed in relation to age in normal tissue. The 4/6-sulfate ratio of the galactosaminoglycans was increased, whereas the proportion of iduronic acid was markedly decreased. □ *High-performance liquid chromatography; polysaccharides*

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The temporomandibular joint (TMJ) has an interposing disk between the temporal component and the mandibular condyle. The function of the disk is mainly to distribute the load from mastication within the TMJ and to reduce friction and shearing forces between the joint surfaces (1).

The disk is composed of fibrocartilage, which forms a dense tissue, rich in collagen and with scattered cells. In rabbits the total content of glycosaminoglycans (GAG) in disk tissue is only about 10% of what is normally found in hyaline cartilage (Unpublished observations). Furthermore, the dominant GAG of rabbit TMJ disks is dermatan sulfate (DS), whereas only minute amounts of chondroitin sulfate (CS) and other disaccharides are present (2). The content and composition of GAGs are thus somewhat like those of the tendons. The histologic and histochemical characteristics of the disk in humans resemble those in rabbits (3–6), but the content of GAGs has not been determined and characterized by biochemical means.

The quality and mechanical properties of articular cartilage, such as the disks, depend on its matrix constituents. The collagen fibers form a dense meshwork that gives the tissue mechanical strength, while the non-collagenous matrix, mainly proteoglycans and GAGs, provide a high-charge density resulting in a correspondingly high osmotic pressure. This combination of meshwork and osmotic pressure, striving to expand the tissue, provides an organ that is able both to absorb shock and to distribute loading forces.

Like other synovial joint cartilages, the TMJ is prone to develop osteoarthosis (OA) when submitted to an excessive load, as happens during bruxism and unilateral chewing (7) or if teeth are lacking (8). Microscopically, both the disk tissue and the articular hyaline cartilage degenerate during the course of the disease, and eventually the underlying bone may be affected (9). Some inflammation of the capsule and disk attachments may be seen, although OA is considered to be a mainly non-inflammatory disease (10).

In hyaline articular cartilage OA is associated with the disintegration of the collagen network and the subsequent fibrillation of the tissue. A simultaneous degradation and loss of otherwise entrapped proteoglycans (PGs) has also been demonstrated (11, 12). These alterations influence the ability of the tissue to retain water, which then affects elasticity and load-distributing properties. Such mechanical deterioration may, in turn, induce cell damage and a further release of proteolytic enzymes. Regardless of which of these processes occurs first—that is, enzyme activation, cell damage, or matrix degradation from other causes—they all contribute to the severe deterioration in the mechanical properties of the affected tissues. A vicious circle may thus be started with proteolytic enzyme activity, followed by degradation of matrix constituents and changes in the shock-absorbing properties of the tissue, resulting in cell damage and further proteolytic activity.

The observed alterations in GAG content in arthrotic hyaline articular cartilage thus seems to be an integral part of the pathophysiology of the disease. The unaffected fibrocartilagenous TMJ disk tissue, however, has a very different composition, with a much lower content of GAGs, and the role of these substances in relation to arthrosis remains to be demonstrated. As a first step in this direction our aim was to isolate and characterize the GAGs present in the normal and osteoarthrotic human disk tissue of the TMJ.

Table 1. Glycosaminoglycan content in human temporomandibular joint disks (in μg HexN per mg dry weight)

	Normal		Arthrosis	
	Anterior	Posterior	Anterior	Posterior
CS/DS	4.7 ± 1.1	5.2 ± 0.8	1.7 ± 0.8	1.1 ± 0.6
HA	0.5 ± 0.2	0.7 ± 0.4	0.02 ± 0.02	0.2 ± 0.3
HS	0.2 ± 0.2	0.3 ± 0.2	0.1 ± 0.01	0.2 ± 0.3
KS	*	*	*	*
ΣGAG	5.4 ± 1.0	6.2 ± 1.0	1.8 ± 0.8	1.5 ± 0.8

CS/DS = chondroitin sulfate/dermatan sulfate; HA = hyaluronate; HS = heparan sulfate; KS = keratan sulfate; GAG = glycosaminoglycans.

* Less than 0.01 μg HexN/mg dry weight.

Materials and methods

Tissues

Ten virtually unaffected human TMJ disks were obtained at autopsy from five patients—two female and three male. The ages ranged from 17 to 80 years, with a median of 43 years. The autopsies were performed within 36 h after death. The disks showed no macroscopically detectable degenerative changes; they were all biconcave and avascular in the weight-bearing areas. In no case was there any systemic disease affecting the joints or any known trauma to the head. The disks were dissected free of capsular and muscular tissue and divided into one anterior and one posterior part. This dissection was carried out so that the anterior part of the disk would include the thin load-bearing areas of the tissue. The tissue were then frozen in liquid N_2 until analyzed.

Six TMJ disks from five patients—four female and one male—were obtained at TMJ surgery. The ages of the patients with OA ranged from 28 to 59 years, with a median of 47 years. The diagnosis that led to surgery was disk derangement with associated pain and impairment of function. All patients had been treated non-surgically before surgery, comprising splint therapy, occlusal adjustments, and physiotherapy. Local intra-articular injections were not given in any case. All disks extirpated at surgery were macroscopically deformed. None of the disks had a normal biconcave shape, and in most of them the normally thick posterior band of the disk seemed to be dislocated antero-medially over the condyle, with the mandible in closed position, according to observations at surgery. Since the arthrotic disks were all abnormal in shape, it was difficult to identify the original posterior band at dissection. They had also been anteriorly displaced for at least 12 months, and during this period the weight-bearing area had been displaced to the posterior portion. The tissues were dissected and separated into an anterior and a posterior fraction similar to those in the normal specimens, and a sagittal slice from both the lateral and medial portion were taken for histologic examination (10). Only

Table 2. Galactosaminoglycan composition in human temporomandibular joint disks

	Normal		Arthrosis	
	Anterior	Posterior	Anterior	Posterior
Sulfation pattern				
Non-sulfated	0	0	0	0
4-Sulfated	33	32	65	80
6-Sulfated	67	68	35	20
Uronic acids				
Iduronic acid	36	28	12	15
Glucuronic acids	64	72	88	85

The figures express percentages of disaccharides.

disks showing histologically verified degenerative changes were used in the study. The light microscopic changes have previously been described (10).

Chemicals

High molecular weight hyaluronate (HA) standard (Healon®) was obtained from Pharmacia AB (Uppsala, Sweden). CSs were obtained from Sigma (St. Louis, Mo., USA) as their grade-II and 4-sulfated grade-I preparations. Unsaturated disaccharide standards (delta-di-4S and delta-di-6S, respectively) and idose standards were also from Sigma, the latter in an 85% pure preparation. Papain (twice recrystallized), chondroitinase AC, chondroitinase ABC, chondroitin-4-sulfatase, and chondroitin-6-sulfatase were all obtained from Sigma. All other reagents were of analytic grade.

The tissue was digested with papain (13) for 4 h at 65 °C, the digestion buffer containing 0.05 M ethylenediaminetetraacetic acid (EDTA) to decalcify the tissue. The uronic acid containing GAGs was precipitated from these digests by the addition of 4% cetylpyridinum chloride (CPC). For further purification of CPC-precipitable GAGs, the pellet obtained was dissolved in 0.4% CPC and 60% *n*-propanol, whereafter the GAGs were reprecipitated with four volumes of a 9:1 mixture of ethanol and 25% w/v sodium acetate in water (14). After being washed in 99% ethanol and subsequently lyophilized, the precipitate was dissolved in 24 mM Tris buffer, pH 7.5. The CPC supernatant was analyzed for its content of non-precipitable GAGs—that is, keratan sulfate (KS), as described below.

Analytic procedures

To analyze the contents of HA, CS, and DS, an aliquot containing 50–150 μg uronic acid/ml of the GAG preparation was digested overnight at 37 °C with a mixture of chondroitinase AC and chondroitinase ABC, using 0.1 U of each enzyme/ml of the digestion mixture. To measure the contents of HA and galactosaminoglycans (CS and DS, respectively), aliquots of these digests were further digested by the addition of chondroitin-4- and chondroitin-6-sulfatases (0.1 U/ml). The non-sulfated 4,5-unsaturated disaccharides obtained were then analyzed with a high-performance liquid chromatography (HPLC) procedure (15). To determine the position of the sulfate group of CS/DS, another aliquot of the chondroitinase digest was chromatographed at a higher ionic strength and at a higher pH (15) but without preceding sulfatase digestion. The contents of iduronic acid (IdUA) and glucuronic acid (GlcUA) in GAG preparations were determined by means of another HPLC procedure described by Karamanos et al. (16). To analyze the presence of chondroitinase-resistant and CPC-precipitable GAGs—that is heparan sulfate (HS)—this analysis of uronic acids was also applied to chondroitinase digests. Hexosamine contents of the CPC-precipitated GAG preparations were further analyzed by hydrolysis of the samples, using 8 M HCl for 3 h at 95 °C, followed by lyophilization. The dansylated derivatives obtained were chromatographed on a Supelcosil LC 18 column as described (17). The supernatant from CPC precipitation was taken for analysis of its KS content (18). CPC was extracted by repeated washing with isopentyl alcohol. The residues were diluted tenfold and added to 0.5 ml DEAE anionic ion exchange columns. After the column had been washed with 1 ml H_2O followed by 1 ml 0.05 M LiCl and 1 additional ml of H_2O, the KS was eluted with 1.5 ml 6 M HCl. After lyophilization the hexosamine content of this GAG fraction was analyzed for hexosamine contents.

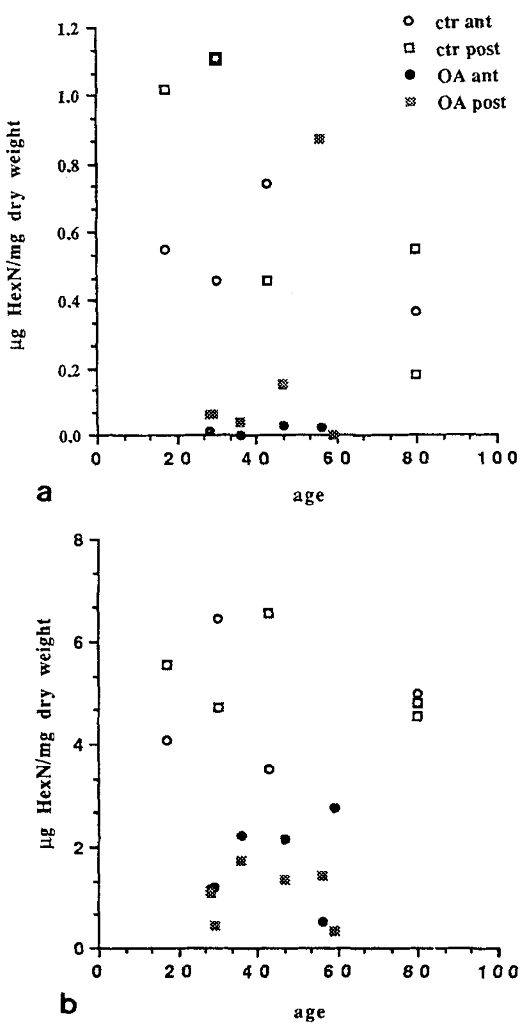

Fig. 1a. Content of hyaluronate in normal and arthrotic temporomandibular joint disk tissue, anterior and posterior portion, in relation to age. 1b. Content of chondroitin sulfate/dermatan sulfate in normal and arthrotic temporomandibular joint disk tissue, anterior and posterior portion, in relation to age.

Results

The normal disk tissue contained an average of 5.4 μg hexosamine/mg tissue dry weight in the anterior part, whereas the posterior portion was somewhat richer in GAGs, with a content of 6.2 μg hexosamine/mg tissue dry weight (Table 1). The galactosaminoglycans (CS and DS) accounted for 90% of the total GAG content in the anterior part and 84% in the posterior part. In these galactosaminoglycans 28% (posterior) to 37% (anterior) of the disaccharides contained iduronic acid, demonstrating the presence of DS. HA composed 10% of the total GAG content. Minor amounts of HS and KS were also detected, the former with an IdUA/GlcUA ratio of 13:87 in all tissue fractions.

The main part of the galactosaminoglycans was sulfated in the 6-position, with a 4/6 ratio in the order of 1:2 in both the anterior and posterior parts (Table 2). The total recovery of CS/DS-derived disaccharides exceeded that of monosulfated ones before sulfatase digestion, which also indicated the presence of some non-sulfated moieties. There were no signs of oversulfation of CS/DS.

The values obtained for the content and composition of GAGs showed some variation with age, as can be seen in Fig. 1. With increasing age there was a decrease of HA contents, whereas changes in CS/DS were less obvious. The older TMJ disk tissue, however, contained CS/DS with a slightly lower 4/6 sulfate ratio.

The total amount of GAGs present in the arthrotic disks was markedly lower than in the normal tissue (Table 1). When estimated on a dry tissue weight basis, the concentration was reduced to 33% or less. This change was most apparent in comparisons of the posterior parts of the disks. The difference between the anterior and posterior tissue fractions was less marked in the OA disks, with a GAG composition similar to that in normal tissues.

Thus the galactosaminoglycans were also the dominant GAGs in the arthrotic disks, smaller amounts of HA and traces of KS and HS being present. The composition of the galactosaminoglycans in arthrotic disk tissue (Table 2) was, however, altered. The proportion of IdUA was reduced to 12%–15% of the CS/DS uronic acid content. The proportion of 4-sulfated disaccharides increased to 65%–80% (anterior and posterior parts, respectively). When related to the total concentration of CS/DS, the ratios indicated a decrease in the content of both kinds of uronic acid, the lowered recovery of IdUA being more prominent. Similarly, the 6-sulfated disaccharides were much lower than normal disk tissue, whereas the change in 4-sulfated disaccharides was much smaller. It was not possible to demonstrate an age-dependent variation in GAG content and composition in the arthrotic disk tissue (Fig. 1). In no case did the age-related changes observed in the normal tissue explain the much larger differences found between normal and affected tissue.

Discussion

The content of GAGs (5.4–6.2 μg hexosamine/mg dry weight) demonstrated in normal TMJ disks was in every case considerably lower than that normally found in hyaline articular cartilages, such as in the hip joint (19). The fibrocartilage of the canine knee meniscus also has a higher GAG content, amounting to 20 μg uronic acid/mg dryweight (20), whereas in bovine tendon tissue GAGs occur at a concentration about half that of the present findings in TMJ disks (21). The finding that CS/DS is the major GAG in TMJ disk tissue is not specific for cartilage but is a common finding in several connective tissues. DS is present primarily in fibrous tissues, such as fibrocartilage and tendons. The substantial proportion of IdUA in this GAG fraction is remarkably different from the trace amounts found in hyaline articular cartilage (22), which emphasizes the fibrous nature of the TMJ disk. The proportion of IdUA observed is similar to that in human menisci (27% (23)), although higher values have been reported in the bovine tendon (21).

The present finding that HA contributes 10% of the total GAG content is only slightly higher than that in hyaline articular cartilage (8% (24)) and in the fibrocartilage of the meniscus (7% (20)), whereas considerably lower values have been reported in the bovine tendon (2% (21)). The GAG composition of TMJ disk tissue thus lies between that of hyaline cartilage and tendon tissue but is similar to that in other fibrocartilages, such as in the meniscus of the knee.

Age-related changes in hyaline articular cartilage have previously been shown not to involve the content so much as the composition of its GAGs. In hip and knee joint cartilages the relative proportion of CS decreases with a simultaneous increase in KS. The CS become highly sulfated, whereas the 4/6-sulfate ratio decreases (25). Some authors have reported that the tissue content of HA increases with age (26), while others have failed to verify this (25). The TMJ disk tissue differs from these hyaline cartilages in that the total content of HA decreases significantly with age, whereas the CD/DS is less affected. The severe biochemical alterations observed in arthrotic disk tissue, however, cannot be explained by these more subtle age-related changes. The posterior and anterior tissue fractions from both normal and arthrotic disks did not differ much in the concentration of total GAGs or in their composition, with the exception of the content of DS-derived IdUA, which was somewhat higher in the anterior load-bearing area of the normal disk.

The decrease in the GAG content of arthrotic disks is in good accordance with previous histochemical findings (4). The findings also show some similarities to those of Adams et al. (27), who studied experimental arthrosis in dogs. After sectioning of the crucial ligament they found an initial loss of GAGs from the meniscus fibrocartilage, but this decrease reverted to normal, and no changes in the ratio of 4/6 sulfate and IdUA/GlcUA could be demonstrated. The present extensive changes in the arthrotic TMJ disks in humans, compared to those of the meniscus of the dog, may reflect a more long-standing, chronic state of the disease.

Naturally occurring arthrosis of hyaline articular cartilage is different from that of TMJ disks, since the former is not associated with any significant changes in GAG concentration (28). Some experimental studies show effects on the GAG contents, but the various models seem to be inconsistent in this respect (19, 29, 30).

The GAGs of TMJ disk tissue differ from those of cartilage and tendon tissue and show some characteristics typical of both these tissues. This intermediate pattern is in good accordance with the morphology of this highly fibrous cartilage. The difference between TMJ disk tissue and hyaline articular cartilage, with regard to changes in GAGs with osteoarthrosis, is substantial. This incongruence may depend on the composition of the matrices involved. An initial loss of GAGs in hyaline cartilage (25) may affect the mechanical properties of the tissue to such an extent that it deteriorates, leaving only less altered remnants of the tissue. The tissue cohesiveness of fibrocartilage may be higher due to its higher content of collagen. When an equivalent loss of GAGs then occurs (27), the tissue may remain macroscopically intact, enabling the resulting alteration in the content and composition of GAG to be demonstrated.

For a better understanding of the role of GAGs it is important to study them in their macromolecular organization—that is, as PGs—and to elucidate further the properties of these complexes. The use of autopsy material hampers such studies. To facilitate further investigations in this respect, an experimental animal model of TMJ disk arthrosis is at present being worked out in our laboratory.

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