Metabolic turnover of sulfated glycosaminoglycans and proteoglycans in rabbit temporomandibular joint cartilages with experimentally induced osteoarthrosis

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Osteoarthrosis-like changes were induced by means of experimental disk perforation in the right temporomandibular joint of rabbits. The turnover of proteoglycans and glycosaminoglycans was studied 16 weeks later, using $^{35}SO_4$. Tissues were sampled 1 day and 7 days after injection of the sulfate. The corresponding tissues from the left untreated joint were used as controls. After isolation of the glycosaminoglycans the incorporation of $^{35}SO_4$ was estimated by scintillating counting. The extracted proteoglycans were analyzed, using gel electrophoresis, and the distribution of radioactivity was determined by autoradiography, followed by densitometry. Both the synthesis and rate of degradation of the proteoglycans were increased in the experimental disk, compared with those of the control. The net result of these metabolic changes seemed to be losses of small proteoglycans, whereas a slow increase in the number of larger ones may have occurred. The turnover rates of 4– and 6-sulfate increased, although their ratio remained unchanged at this stage of the osteoarthrosis-like process. In the condylar cartilage the turnover of large and small proteoglycans was also increased. The increase was most marked among those containing 6-sulfated galactosaminoglycans. The results concerning the experimental condylar cartilage indicated a decrease in the largest proteoglycan population, whereas the proportion of small proteoglycans was increased. □ *Biochemistry; temporomandibular joint disease*

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The cartilage of the condyle and the glenoid fossa of the temporomandibular joint (TMJ) consists of a deeper hyaline portion covered by a fibrous lining in which the collagen bundles run parallel to the joint surface (1). It is postulated that the mesenchymal cells in the border between these two portions play a role in regeneration and rapid adaptation of the joint surface when the load changes (2). The deeper portion of the cartilage resembles the epiphyseal cartilage because it shows hypertrophy of chondrocytes and mineralization of cartilage matrix near the osteochondral junction (3). The interposing disk is composed of a cartilage that is extremely fibrous. In the central portion of the disks very dense collagen bundles lie mostly parallel to the disk surface, whereas in the periphery they form an interwoven pattern. A few flattened cells are seen throughout the normal disk tissue (4).

Proteoglycans (PG) are important components of both the articular cartilage and the disks, although they are not so abundant as in most other articular cartilages. The total estimated uronic acid content of normal rabbit condylar cartilage is only about 5 μg/mg dry tissue, whereas the rabbit TMJ disk contains only about 2 μg uronic acid/mg dry tissue (5). The condylar cartilage consists mainly of large PG molecules, representing 72% of the total uronic acid content (6). This is also in accordance with the glycosaminoglycan (GAG) composition of this tissue, which is dominated by chondroitin sulfate

(CS), although smaller amounts of hyaluronate (HA) and keratan sulfate (KS) are also present (6). The PGs in the disk are mainly small, and the GAG composition is more like that found in soft connective tissues. Thus, galactosaminoglycans containing iduronic acid indicates that there is an abundant amount of dermatan sulfate (DS) and only trace amounts of KS (7).

Osteoarthrotic changes in joint components are common in the TMJ, as evidenced by autopsies showing osteoarthrotic lesions in about 22–38% of the joints examined (8–10). The etiology of TMJ osteoarthrosis is considered to be multifactorial, important factors being increased load or shearing forces contributing to the destruction of normal joint tissue, or impairment of joint components, leaving the joint more vulnerable to normal wear. In response to this, there may be regeneration of the matrix, reinforcing or repairing the tissue. However, if such compensatory responses are insufficient—that is, if the stress on the joint components exceeds these compensatory reactions—irreversible damage will occur (11).

Early osteoarthrotic changes in the TMJ have been studied experimentally in rabbits. We have described how such lesions may be induced by perforation of the TMJ disk (5). After 16 weeks histologic and biochemical findings suggest that this is followed by regeneration. The disk shows signs of new tissue formation of foci of mineralization cartilage with a hyaline appearance. These morphologic features are accompanied by biochemical changes. The total GAG content per milligram dry tissue weight increases, as does the proportion of glucoronic acid in the disk galactosaminoglycans. Concurrently with the hyaline morphology, the proportion of large PGs increases. At the same time the condylar cartilaginous head also shows morphologic changes characterized by a less distinct border between the fibrous and hyaline portions. Here the GAG content is also increased; the increase, however, is mainly confined to the small PG population.

The present study was undertaken to establish whether the increased GAG content in these matrices is due to a forced production, a hampered destruction, or a combination of these processes. This metabolic background was also studied in relation to the different populations of PGs in the tissue.

Materials and methods

Experimental model

Ten male adult New Zealand white rabbits were used in the study. An operation on the right TMJ created a perforation in the posterolateral part of the disk, as earlier described (5). The contralateral joint was left untreated as a control. After 16 weeks an intravenous injection of 10 mCi/kg body weight of $^{35}SO_4$ carrier-free sodium sulfate (NEX-041H, DuPont de Nemours, Dreieich, Germany) was given to each rabbit. The rabbits were killed, five of them after 24 h and the other five after 168 h (1 week). Disk tissue adjacent to the perforation on the experimental side of the corresponding disk area on the contralateral side were dissected and collected, as was the condylar cartilage from the load-bearing area on both contralateral condyles. The experimental and control tissues were pooled and frozen in liquid nitrogen. An amount of tissue corresponding to that which could be obtained from three animals was then dried in cold acetone for the GAG analyses, whereas the remaining tissues were used to study PGs. Synthetic rates were directly estimated from the amount of incorporated isotope, whereas degradation rate constants and half-life times were calculated from the difference between days 1 and 7, assuming first-order kinetics.

Isolation of GAGs

After dehydration and defatting in acetone, the dried tissue fractions were digested with papain in an ethylenediaminetetraacetic acid (EDTA)-containing phosphate buffer at pH 7 for 4 h in 65°C (12). The GAGs were subsequently precipitated by 4% cetylpyridinium (CPC), dissolved in 0.4% CPC/60% *n*-propanol, and reprecipitated with 90% ethanol containing 2.5%

sodium acetate (13). The precipitates were then dissolved in 0.3% tris buffer. The galactosaminoglycan-containing precipitates were then dissolved in 0.3% tris buffer and digested with a mixture of chondroitinase AC and chondroitinase ABC (Seikagaku Kogyo Co., Ltd., Tokyo, Japan) at 37°C overnight (14). The 4- and 6-sulfated disaccharides obtained were fractionated by high-performance liquid chromatography (HPLC) using an NH_2 column and eluted with 5 mM Na_2SO_4/10 mM sodium acetate at pH 5.0 (15). The fractions were collected for subsequent scintillation counting, which was performed by adding 10 volumes of Aquasol-2 (New England Nuclear, Boston, Mass., USA) and using a 1214 Rackbeta liquid scintillation counter (LKB Wallac, Uppsala, Sweden).

For analysis of the $^{35}SO_4$ incorporation in the different PG fractions, the frozen tissues were cut in a microtome into 50-μm-thick sections. The tissue was then freeze-dried, and the PGs were extracted with 4 M guanidine hydrochloride (GuHCl) containing protease inhibitors (16). The PG extracts were analyzed by gel electrophoresis (17). This involves Alcian blue precipitation of PGs in a 4M GuHCl solution at pH 1.5. The precipitates were then redissolved in a GuHCl-*n*-propanol mixture, and the PGs were reprecipitated by adding further propanol. All the PGs were analyzed by subsequent spectrophotometry of the Alcian blue-containing propanol supernatants. The PG precipitates were dissolved in a sodium dodecyl sulfate (SDS)-containing electrophoresis buffer, and the electrophoretic mobility was monitored by separation on 1.2% agarose gels at 90 V for 1 h (18). The distribution of radioactivity was then detected by autoradiography, followed by densitometry.

Results

The various PG populations and those newly synthesized are readily demonstrated with the electrophoretic procedure used (Fig. 1). In the normal disk tissue the large and small PG populations differ considerably with regard to turnover times (Table 1). The large

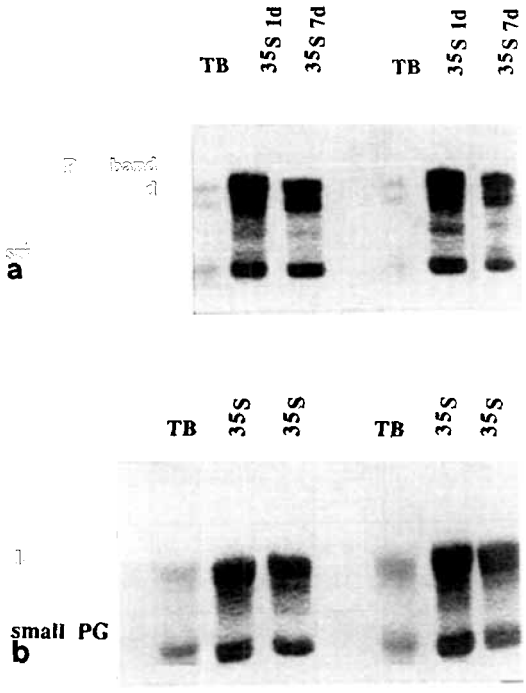

Fig. 1. Condylar cartilage (1a) and disk tissue (1b) proteoglycan populations as separated by electrophoresis. Distribution of polysaccharides demonstrated by toluidine blue (TB) staining (only gel from 1 day after injection shown) and incorporated $^{35}SO_4$ by autoradiography on the same gels.

PGs are degraded with a biologic half-life of 6.3 days, whereas the small PGs are degraded with a biologic half-life of at least 60 days, the turnover rate being very slow. The average turnover of large PGs in the normal condylar cartilage was much the same as for the large PGs in the disk tissue (Table 2). However, the two large PG populations differ in this respect, the largest being associated with the lowest turnover rate. In the small PG population the disk and the condylar cartilage show different turnover rates, the half-life in the latter being only 6 days. Analysis of the GAG fraction indicates that the 6-sulfated galactosaminoglycans have slightly higher metabolic values in the disk tissue, whereas the condylar cartilage shows similar values for these two disaccharides (Table 3).

Table 1. Incorporation and degradation of $^{35}SO_4$ in the temporomandibular joint disk proteoglycans

	Specific activity, cpm/μg uronic acid	Sulfate incorporation, cpm/mg dry tissue	$t_{\frac{1}{2}}^*$, days	Rate of synthesis relative to control*	Rate of degradation relative to control*
Operated disk					
Large PG	1245	1294	8.3	1.2	0.9
Small PG	411	481	6.2	2.8	>4
Σ PG	795	1775	8.7	1.4	1.3
Control disk					
Large PG	3292	1092	6.3	—	—
Small PG	193	175	>60	—	—
Σ PG	988	1267	12	—	—

* Estimation assuming first-order kinetics.

Table 2. Incorporation and degradation of $^{35}SO_4$ in the temporomandibular joint condylar cartilage proteoglycans

	Specific activity, cpm/μg dry tissue	Sulfate incorporation, cpm/mg dry tissue	$t_{\frac{1}{2}}^*$, days	Rate of synthesis relative to control*	Rate of degradation relative to control*
Operated cartilage					
Large PG, band 1	1955	6832	5.2	2.0	4.3
Large PG, band 2	6083	7019	3.0	2.3	2.9
Σ large PG	2919	13,567	4.3	2.2	3.3
Small PG	743	2913	11	1.9	1.0
Σ PG	1882	16,129	5.8	2.0	2.2
Control cartilage					
Large PG, band 1	1452	3364	11	—	—
Large PG, band 2	3861	3043	4.0	—	—
Σ large PG	2035	6319	7.0	—	—
Small PG	1032	1553	6.0	—	—
Σ PG	1718	7924	6.3	—	—

* Estimation assuming first-order kinetics.

The metabolism of the matrix PGs is considerably changed 16 weeks after the disk perforation. In the disk tissue, incorporation of sulfate is increased and is mainly expressed among the small PGs, which have a synthesis rate 2.8 times greater than the corresponding controls (Table 1). The small PGs are also degraded at a higher rate than the large ones, and the turnover is considerably greater than that of the small PGs in normal tissue. The turnover of the large PG population shows only minor deviation as compared with the corresponding controls. The net result thus seems to be losses of small PGs, whereas there may be a slow increase in the number of large ones. The galactosaminoglycans show an increased sulfate turnover in both the 6-sulfated and 4-sulfated moieties, the turnover rate being greatest for the 4-sulfated ones. The net result of this 4/6-sulfate turnover seems to be balanced just as in the control tissue (Table 3).

The metabolism of GAG and PGs is also changed in the experimental condylar cartilage. The synthetic rates of both small and large PGs are increased (Table 2). Both large PG populations showed a faster degradation than that in the control tissues, whereas the turnover time of the small ones increased when comparing anabolic and catabolic rates. The figures obtained indicate a net decrease in the largest PG population, whereas in this condylar tissue the small PGs increased. The increased turnover is most marked among PGs containing 6-sulfated galactosaminoglycans. The relation between the 4- and 6-sulfated galactosaminoglycans, however, does not seem to be affected (Table 3).

Table 3. Incorporation and degradation of $^{35}SO_4$ in the 6- and 4-sulfated (6-S and 4-S) galactosaminoglycans in temporomandibular joint tissue

	cpm/μg UA*		
	1 day	1 week	$t_{\frac{1}{2}}$
Operated disk			
6-S	1564	987	9.0
4-S	1244	848	11
Control disk			
6-S	1197	836	12
4-S	887	687	16
Operated cartilage			
6-S	4238	2063	5.8
4-S	2980	1774	8.0
Control cartilage			
6-S	3632	1673	5.4
4-S	3647	1969	8.0

* UA = uronic acid.

Discussion

The present experimental model for studying osteoarthrosis is based on the idea that an uneven joint surface would result in a vicious circle encompassing changed load, cell damage, and release of lytic enzymes. Our previous studies (5, 6) have shown distinct morphologic and biochemical alterations in the tissue. The tissue contents of PGs and GAGs are greatly changed concurrently with the development of this osteoarthrosis-like condition, showing the importance of these substances.

The slow turnover time of the small PG population in the normal disk tissue must be considered with regard to the fibrous nature of this tissue. These PGs may represent the so-called decorins and biglycans, small PGs that have been associated with collagen synthesis and structure (for further references see Ref. 19). The different turnover rates for these larger PGs indicate a different role for this molecule in the tissue. Similarly, in the normal condylar cartilage the differences obtained also reflect different functions in the tissue. Here, the presence of small, collagen-related PGs together with large aggrecan-like ones may reflect the different zones of fibrous connective tissue outlining the articular surface and the underlying cartilage.

The perforation of the disk apparently leads to reactions in the affected tissues which, 16 weeks later, results in a condition with an osteoarthrosis-like morphology (5). The metabolic activity in both the disk and condylar tissues seems to reflect a condition seen when the adaptive ability of the tissue is challenged by degenerative processes. In the large PG fraction in the disk there seem to be simultaneous increases in both the syn-

thesis and the degradation rates, indicating a metabolic balance. This may imply that the cells synthesizing large PGs are coping with the stress induced in the tissue and that they have adapted to the new situation. In the fraction of small PGs, however, the rate of degradation is greater than the moderately increased synthesis. The result is a relative increase in the number of large PGs. The reparative processes thus seem to fail, as appears to occur in early osteoarthrosis (11). However, changes observed differ considerably from those described in advanced lesions in human TMJ disks, where a more consistent loss of GAGs was demonstrated (20). A decrease in histochemical reactivity of GAGs has also been demonstrated in human osteoarthrotic disks by Kopp (21). In consequence of the increased synthesis, the total PG content is higher in the experimental disks, and the proportion of large PGs is increased in relation to that of the control tissue. The increased proportion of large PGs may result from a change in the phenotypic expression of the experimental disk tissue. Close to the perforation the matrix appears somewhat more basophilic, and at a larger distance from the rim we found foci of a more chondroid appearance, similar to, for example, callus tissue. The area containing these foci was not, however, included in the portion of the disk tissue that was analyzed.

The content and composition of PG populations are also altered in the condylar cartilage. The increase in the total content is due to the increased synthesis of both small and large PGs, which may constitute attempts at reparation also in this tissue. The histochemical indication of a decreased GAG content in osteoarthrotic areas of human condylar cartilage (22) may thus reflect a later stage in the osteoarthrotic process. The faster degradation of the largest PG population found in this study indicates that the reparative processes are here insufficient to maintain the balance. However, it is not possible to say whether the changes occur homogeneously throughout the condylar cartilage or whether they merely result from an increase in the proportion of the fibrous surface layer.

The design of the present study does not enable us to determine possible inter-individual variations. However, the two groups studied (16 weeks and 17 weeks, respectively, after operation), each group consisting of five animals, showed virtually the same pattern in the toluidine blue-stained electrophoretic gels.

In conclusion, this early stage of experimental TMJ osteoarthrosis is associated with an imbalance between degeneration and repair. There is thus increased degradation of PGs in both tissues studied and a current compensatory increase in PG synthesis. This reparatory process is for some of the PG populations insufficient to compensate for the increased degradation, explaining an eventual loss of matrix.

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