Proteoglycan fragments in rabbit joint fluid correlated to arthrosis stage

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Proteoglycan fragment concentrations in joint fluid of rabbit knees with various degrees of secondary arthrosis after meniscal resection, meniscal substitution, and anterior cruciate ligament transection, were related to the gross appearance of the articular cartilage. Knees with normal cartilage were distinguishable by the concentration of proteoglycan fragments in joint fluid from knees with arthrosis. We found a correlation (*r* 0.61) between increasing arthrosis and increasing concentrations of proteoglycan fragments.

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Concentrations of proteoglycan fragments in synovial fluid are inversely related to the degree of radiographic joint destruction in patients with rheumatoid arthritis (Saxne et al. 1985). A similar relationship was found in human arthrosis (Dahlberg et al. 1992). However, it is difficult to recruit patients in the earliest stage of the disease, radiographic changes only represent a record of past destructive disease, and arthroscopic staging of cartilage changes is difficult.

We have determined the concentrations of proteoglycan fragments in joint fluid from rabbit knees with different degrees of secondary arthrosis.

Material and methods

Arthrotomy (sham-operation), meniscectomy, or meniscal substitution with a prosthesis (Sommerlath and Gillquist 1992) was performed in 48 skeletally mature New Zealand white rabbits; in 36 of the rabbits the anterior cruciate ligament was transected simultaneously (Table 1). The prostheses were manufactured by Stryker BV, Uden, The Netherlands. In addition, control samples were obtained from both knees of 13 age-matched animals, where no previous knee surgery had been performed.

All operations were performed using intravenous anesthesia. Both knee joints were opened with anterior and posterior capsular incisions. The prosthesis was implanted after meniscectomy. Sham-operated knees had only capsular incisions, without damage to ligaments or intraarticular structures.

After surgery the rabbits were allowed to move freely in their cages (area 0.5 m^2). 18 of the animals with ligament transection were killed at 6 weeks, and the remaining animals at 3 months. Immediately after death, 2 mL of sterile sodium chloride solution was injected into the knee joint, and the knee was taken 10 times through a full range of motion. As much as possible of the joint fluid was then aspirated and the sample stored at –20 °C until analyzed. The hind limbs of the rabbits were disarticulated at the hip joint and stored at a similar temperature.

The concentration of proteoglycan fragments in the joint fluid was analyzed as sulfated glycosaminoglycan spectrophotometrically by the dimethylmethylene blue method (DMMB-method; Farndale et al. 1982) and by precipitation of sulfated glycosaminoglycan containing proteoglycan fragments with alcian blue

Table 1. Surgical treatments of the animal knees

	Right n	Left n	Joint fluid[a]
Controls (n 13)	13	13	1.0 *0.4*
Intact ligaments (n 12, killed at 3 months)			
Sham-operation		8	1.2 *0.3*
Meniscectomy		4	1.2 *0.5*
Prosthesis	12		1.1 *0.4*
ACL-deficiency (n 18, killed at 6 weeks)			
Intact menisci	4	4	1.6 *0.2*
Meniscectomy		14	1.3 *0.4*
Prosthesis	14		1.2 *0.5*
ACL-deficiency (n 18, killed at 3 months)			
Intact menisci		10	1.0 *0.5*
Meniscectomy		8	1.1 *0.3*
Prosthesis	18		0.9 *0.4*

[a]mL joint fluid aspirated after injection of 2 mL sodium chloride. Mean *SD*

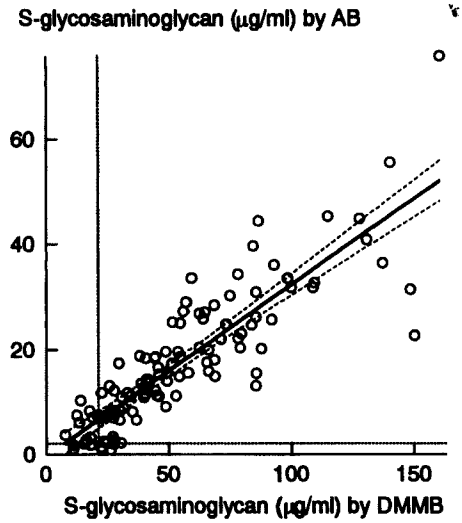

Figure 1. Linear regression of the concentration of sulfated glycosaminoglycan in synovial lavage fluid measured by the alcian blue or dimethylmethylene blue method. Dotted lines represent mean concentrations in control group. Dashed lines represent 95 percent confidence interval for regression line.

(AB-method; Björsson 1992). For the latter procedure, synovial fluid samples were mixed with an equal volume of 8 M guanidine-HCl, detergent added, and the proteoglycans precipitated at pH 1.5 by the addition of a solution of alcian blue. The final concentration of guanidine-HCl was 0.3 M. The precipitates were collected by centrifugation, washed and dissolved, and their absorbance measured at 600 nM. Chondroitin sulfate (Sigma C4384) was used as standard in both methods. The correlation coefficient between the proteoglycan concentration values obtained with the 2 different methods was 0.87 (AB = –0.3 + 0.33 × DMMB; Figure 1). Due to the presence of guanidine and detergent and the low pH, the AB-method was less prone to interference by, e.g., protein in the synovial fluid.

After thawing, the knee was opened from the lateral side, and rinsed. The femoral and tibial condyles were freed from all soft tissue and were inspected under a dissection microscope. The degree of cartilage destruction of both femoral and tibial condyles was assessed, using a modification of Mankin's histologic classification (Sommerlath and Gillquist 1992): Grade 0, normal cartilage; Grade 1, fibrillation and surface irregularities; Grade 2, pannus formation and surface irregularities; Grade 3, superficial cleft formation; Grade 4, deep, but localized clefts to bone; Grade 5, larger surface defects to bone; and Grade 6, complete loss of cartilage on the load bearing area. The evaluation of the cartilage was done without knowledge of proteoglycan concentrations in joint fluid (Table 2).

The arthrosis score was related to the surgical procedure: in knees with intact ligaments meniscectomy caused more arthrosis than did the sham-operation. Meniscal substitution resulted in less arthrosis than did meniscectomy, but more than in sham-operated knees. Transection of the anterior cruciate ligament caused more arthrosis than did the operations with intact ligament. Ligament transection already caused at 6 weeks the highest arthrosis scores (Grades 5 and 6). Between the 6- and 12-week specimens, no differences were found; the results of the arthrosis score are, therefore, listed together (Table 2). The 12-week specimens, however, appeared to have a larger amount of cartilage loss, a higher degree of bone destruction, and a larger osteophyte formation.

For statistical calculations the maximum arthrosis in each joint was used as an expression of the stage of

Table 2. Arthrosis and concentrations of proteoglycan fragments (µg/mL) in joint fluid after different surgical treatments. Mean *SD*

Surgical method	n	Maximum score of arthrosis (0–6) median (range)	Proteoglycan fragments	
			DMMB-method	AB-method
Intact ligaments				
Controls	26	0 (0)	21 *7.8*	2.1 *0.8*[c]
Sham-operation	8	0 (0–4)[a]	21 *4.9*	7.4 *0.6*
Meniscectomy	4	4 (2–5)	25 *9.7*	8.0 *2.8*
Prosthesis	12	2 (1–5)[b]	42 *18*	12 *4.6*
ACL-deficiency[d]				
Intact menisci	18	5 (0–6)	78 *39*	27 *17*
Meniscectomy	22	5 (3–6)	67 *26*	26 *10*
Prosthesis	32	5 (2–6)	69 *32*	20 *8.2*

[a] Less arthrosis than with other treatments ($P < 0.01$).
[b] Less arthrosis in paired comparison than in knees with meniscectomy ($P < 0.05$).
[c] Lower concentrations of proteoglycan fragments than with the other alternatives ($P < 0.0001$).
[d] Higher concentrations of proteoglycan fragments than in knees with intact ligaments ($P < 0.01$).

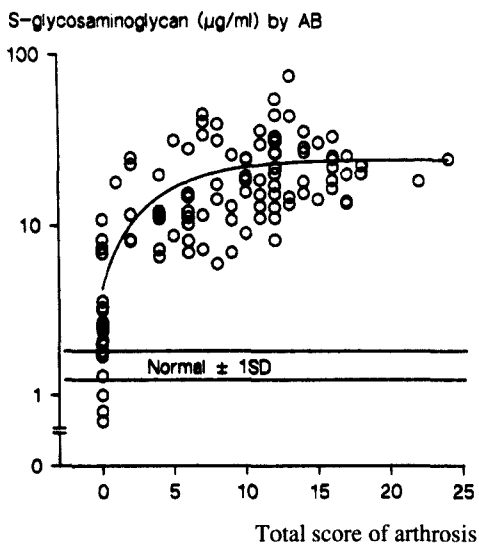

Figure 2. Correlation between the concentration of sulfated glycosaminoglycan in lavage synovial fluid and the total score of arthrosis; x-axis, total score of arthrosis (scale 0–24); y-axis, log concentration of sulfated glycosaminoglycan in joint fluid (µg/mL); *r* 0.49 (AB-method).

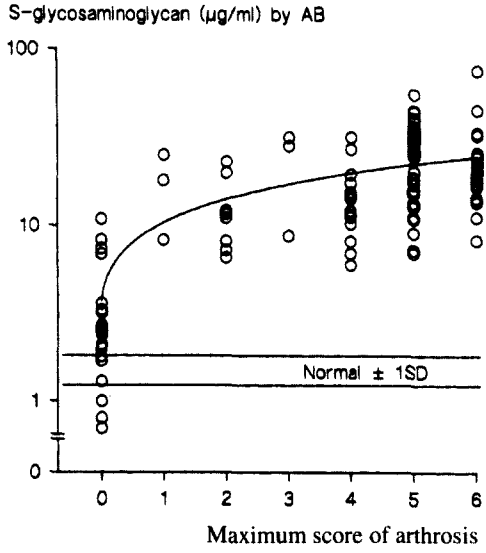

Figure 3. Correlation between the concentration of sulfated glycosaminoglycan in lavage synovial fluid and the maximum score of arthrosis in each joint; x-axis, maximum score of arthrosis (scale 0–6); y-axis, log concentration of sulfated glycosaminoglycan in joint fluid (µg/mL); *r* 0.61 (AB-method).

joint disease. Furthermore, the different degrees of arthrosis of the 4 condyles in each joint were accumulated to give a total score. The maximum and total scores of arthrosis were then correlated to the concentration of proteoglycan fragments in the joint fluid. In order to define sensitivity and specificity of the method, arbitrary levels of proteoglycan fragment concentrations of 30 µg/mL (DMMB-method) and 11 µg/mL (AB-method) were used to distinguish between normal and pathological concentrations in arthrosis.

A paired comparison was made between meniscal replacement and the opposite knee with regard to arthrosis and the increase in proteoglycan fragments. The Student's paired *t*-test was used for continuous variables (concentrations of proteoglycan fragments in joint fluid) and the Wilcoxon signed rank test for categorical data (arthrosis scores) on a 5 percent significance level. Differences in concentrations of proteoglycan fragments and arthrosis between knees from different animals were calculated by unpaired testing. For continuous variables ANOVA was used. In comparisons yielding a significant difference a post-hoc *t*-test was used. For categorical variables the Mann-Whitney U-test was used. The obtained *P*-level was adjusted downwards according to the number of multiple comparisons to compensate for the increased probability of Type 1 error. A significance level of $P < 0.01$ and $P < 0.005$ in some cases was, therefore, required.

Results

Using the AB-method sham-operated differed from control knees by higher concentrations of proteoglycan fragments in joint fluid (Table 2). Overall the concentration of proteoglycan fragments increased with the severity of arthrosis. In the total material, including the non-operated controls, the correlation between the total arthrosis score and concentrations of proteoglycan fragments in joint fluid was non-linear in semilogarithmic plots with correlation coefficients of *r* 0.53 and 0.49 for the DMMB- and AB-method, respectively (Figure 2). A relationship was likewise found between the maximum score of each knee and the concentration of proteoglycan fragments (*r* 0.61; Figure 3). Knees with intact cartilage had a lower concentration of proteoglycan fragments in the joint fluid than had knees with arthrosis. Knees with Grades 5 and 6 arthrosis (maximum score) had higher concentrations than had knees with Grades 2 and 4 arthrosis, but did not differ significantly from knees with Grades 1 and 3 arthrosis. Between knees with Grades 1 to 4 arthrosis no differences were found (Table 3, Figure 3).

The sensitivity of the DMMB-method for indicating arthrosis by elevation of proteoglycan concentration was 87 percent and the specificity 88 percent. The AB-method had an 86 percent sensitivity and a 100 percent specificity.

Table 3. Arthrosis and concentration of proteoglycan fragments (μg/mL). Mean *SD*

Maximum score of arthrosis (0–6)						Total score of arthrosis (0–24)					
Score	n	DMMB-method		AB-method		Score	n	DMMB-method		AB-method	
0	32	22	*7.5*	3.6	*2.8*[a]	0–4	45	28	*15*	7.1	*6.4*[a]
1	3	51	*19*	17	*8.5*	5–8	20	58	*35*	20	*13*
2	10	41	*20*	12	*5.3*[b]	9–12	30	68	*34*	22	*11*
3	3	67	*34*	23	*13*	13–16	19	72	*29*	27	*15*
4	15	46	*25*	15	*7.2*[b]	17–20	6	79	*39*	20	*4.9*
5	36	70	*34*	24	*12*	21–24	2	64	*28*	22	*4.5*
6	23	75	*35*	24	*14*						

Maximum score: knees are distributed according to the highest degree of arthrosis grades 0–6 found on 1 of the condyles.
Total score: to enable classification of global joint arthrosis the different degrees of arthrosis found on the 4 condyles in 1 joint are cumulated, scale 0–24.
[a]Lower concentration of proteoglycan fragments than in all other groups $P < 0.0001$.
[b]Lower concentration of proteoglycan fragments than in more advanced arthrosis—degrees 5 and 6, $P < 0.01$ and less.

Discussion

The rabbit knee is a commonly accepted model to study arthrotic changes in the cartilage. Meniscectomy may induce cartilage ulcera after 2 weeks (Dunham et al. 1985), and transection of the cruciate ligaments was used to study advanced stages of arthrosis (Hulth et al. 1970). We found only 6 weeks after ligament transection the highest degrees of arthrosis, according to our score. Apparently the score focused more on the qualitative differences and did not sufficiently classify the amount of cartilage loss which resulted in an insufficient differentiation in advanced arthrosis. For the representation of the early stages of arthrosis, we included animals with the medial meniscus substituted with a prosthesis because we knew from previous experiments that such knees usually had a lesser degree of arthrosis than those with meniscectomy only (Sommerlath and Gillquist 1992).

In humans increased concentrations of proteoglycan fragments in joint fluid have been noted in association with acute injury to the cruciate ligaments and menisci and also in post-traumatic and primary arthrosis (Lohmander et al. 1989, Dahlberg et al. 1992). Concentrations of proteoglycan fragments decrease with advancing joint destruction in rheumatoid arthritis and arthrosis (Saxne et al. 1985, Dahlberg et al. 1992). However, many of the patients included in these studies already had an established, even advanced, joint disease with bone destruction. It was suggested that the decreasing concentrations partly reflected a decreasing cartilage mass in the joint (Dahlberg et al. 1992). Therefore, the emphasis in these studies was on the later phases of the condition. In contrast, our experiment focused also on the early stages of secondary arthrosis where the need is greatest for methods to diagnose, monitor, and prognosticate the condition.

We suggest that the elevated concentrations of proteoglycan fragments in joint fluid at all degrees of secondary arthrosis in our rabbits represent the rising part of the curve that is lacking in the human studies. The concentration of proteoglycan fragments in joint fluid was more closely related to the maximal severity of cartilage destruction than to the total distribution of arthrotic cartilage surfaces in each joint. This was probably explained by the fact that comparably low concentrations of proteoglycan fragments were found in the few cases with complete loss of cartilage in both compartments. This stage would then correspond to clinical cases with higher degrees of radiologic arthrosis.

With the AB-method, knees which had undergone sham-operation differed from controls. The increased concentrations in sham-operated knees, without correlate in macroscopic cartilage degeneration, may reflect beginning changes in the cartilage matrix and synovitis (Sommerlath and Gillquist 1992). The AB-method proved more sensitive than the DMMB-method in the lower concentration range, probably because of less interference by synovial fluid protein using this particular method (Björsson 1992).

The marked increase in the release of proteoglycan fragment to joint fluid at early stage arthrosis was responsible for the high sensitivity and specificity of this method. However, cartilage in different stages of arthrosis seemed to release similar large amounts of proteoglycan fragments, and these conditions could, therefore, not be distinguished.

Acknowledgements

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