Elevated levels of synovial fluid PLA_2, stromelysin (MMP-3) and TIMP in early osteoarthrosis after tibial valgus osteotomy in young beagle dogs

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We determined the concentration of markers in cartilage and synovium metabolism in the synovial fluid (SF) of the knee of young beagle dogs with slowly progressive osteoarthrosis. Osteoarthrosis (OA) was induced by a tibial 30° valgus osteotomy to the right hindlimb of 16 dogs. The contralateral knee served as control. The animals were killed 7 (group I) and 18 months (group II) after operation. The levels in SF of chondroitin sulfate (CS), tissue inhibitor of metalloproteinases (TIMP-1), stromelysin (MMP-3), hyaluronan (HA), and the activity of phospholipase A_2 enzyme (PLA_2) were assayed.

The first microscopic signs of cartilage degeneration were observed 7 months postoperatively and the lesions became more severe, including osteophyte formation during the following 11 months. The synovial fluid level of MMP-3 was higher ($p = 0.04$) at both time-points in the knee joint of the operated hindlimb than in the contralateral joint. On the operated side, 7 months postoperatively, synovial fluid PLA_2 activity was higher ($p = 0.02$) than in the contralateral knee joint, but not 18 months postoperatively. The SF level of TIMP-1 was higher ($p = 0.04$) in the operated joint than in the contralateral joint 18 months after operation. The molar ratio of MMP-3 to TIMP-1 was higher ($p = 0.001$) in group II than in group I.

The changes observed in the concentration of synovial fluid markers in this slowly progressive canine OA model suggest that activation of an inflammation-related process occurs at an early stage of the OA disease induced by unilateral tibial valgus osteotomy.

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Osteoarthrosis (OA) in dogs shows considerable resemblance to the corresponding human condition (Pond and Nuki 1973, Adams and Billingham 1984). To investigate the early mechanisms of OA pathogenesis, experimental animal models must be used. Most OA models entail intraarticular surgery, which has undesirable effects on cartilage and synovium metabolism. One example is the canine OA model, based on transection of the anterior cruciate ligament. This method is well characterized and widely used (Pond and Nuki 1973). However, intraarticular surgery induces bleeding and inflammation in the operated joint, which may affect the tissue processes and confound interpretation of the results (Dingle et al. 1979, Myers et al. 1990). To avoid these drawbacks, extraarticular methods for inducing OA have been tried (Reimann 1973, Johnson and Poole 1988). We have used high tibial 30° valgus osteotomy to induce slowly progressive cartilage degeneration in young beagle dogs (Panula et al. 1997).

Products of articular cartilage and synovial tissue metabolism diffuse into the synovial fluid (SF), where their concentrations have been thought to reflect joint tissue metabolism (Lohmander 1991). Cartilage breakdown products are cleared from the joint by first entering the SF, and there is considerable clinical interest in using their concentrations in SF to assess the severity of joint diseases.

Metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) have been thought to play an important role in cartilage matrix turnover and degradation (Dean et al. 1989). Stromelysin (MMP-3) is known to degrade aggrecan, link protein, and collagen types II, IX, X and XI (Wu et al. 1991). TIMP-1 forms irreversible complexes with the active forms of MMPs with a 1:1 stoichiometry (Murphy 1995). High levels

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of MMP-3 and TIMP-1 have been detected in the SF of patients having RA and OA (Clark et al. 1993, Lohmander et al. 1993), as well as in animal models (Pelletier et al.1988).

Fragments of the degradation (by MMPs) of aggrecan are found in SF. They appear to contain, together with other constituents of aggrecan, a chondroitin sulfate (CS)-rich domain of variable length (Sandy et al. 1992). The level of synovial fluid CS has been considered to reflect the metabolism of aggrecan. The level of aggrecan fragments in SF has been found to increase during the early course of OA and RA (Witter et al. 1987, Lohmander et al. 1993).

Inflammatory episodes of varying duration have been recognized as an integral part of OA (Ehrlich 1984). PLA_2, a key enzyme of the prostaglandin system, generates arachidonic acid, which is further converted into prostaglandins participating in inflammatory reactions (Vadas et al. 1985). Stimulation of degradative enzyme synthesis by chondrocytes has been thought to be related to prostaglandin synthesis via activation of PLA_2 by mechanical trauma (Chrisman et al. 1981). High levels of PLA_2 have been found in SF of RA and OA patients (Kortekangas et al.1994).

A major component of SF, hyaluronan (HA), has an important role in providing joint lubrication and viscoelasticity to SF (Ogston and Steiner 1953). In human and dog OA and RA, the SF concentration of HA is reduced (Dahl et al. 1985, Arican et al. 1994).

We determined changes in synovial fluid markers in the knee of young beagle dogs in the initial phase of slowly progressive osteoarthrosis, induced by tibial valgus osteotomy. Markers were chosen to reflect cartilage matrix metabolism [chondroitin sulfate (CS) in aggrecan fragments], inflammation pathways and synovial and cartilage cell metabolism [(activity of phospholipase A_2 (PLA_2), tissue inhibitor of metalloproteinases-1 (TIMP), stromelysin-1 (MMP-3) and hyaluronan (HA)].

Animals and methods

Animals and operative procedures

16 female beagle dogs of pure breed and same age were purchased from Marshall Farms (North Rose, NY, USA). The dogs were divided into 2 experimental groups, i.e., group I (n 7) and group II (n 9). A slowly progressive osteoarthrosis was induced in the right knee by a method described earlier (Panula et al. 1997). Briefly, a 30° valgus osteotomy, internally fixed with a plate was performed on the right tibia of 16 dogs at the age of 3 months (Panula et al. 1997). The dogs were kept under kennel conditions in 3-dog-fences. The experiment was approved by the Animal Care and Use Committee of the University of Kuopio.

The animals were killed by an overdose of anesthetic 7 months (group I) and 18 months (group II) after operation. The hindlimbs were dissected free from the muscles and the tibial valgus angulation was measured with a goniometer. The color and macroscopic structure of the cartilage and synovial lining were examined.

Cartilage samples

Cartilage specimens were collected from the right and left knees of the operated dogs. 1-mm-thick cartilage slices were cut perpendicularly to the cartilage surface with underlying subchondral bone, using a dentist's drill equipped with 2 cutting discs, separated by a 1-mm spacer. Samples for histology were harvested from the patella, patellar surface of femur and from both weight-bearing condyles of femur and tibia. Mankin's scores were assessed from the histological sections, as described earlier (Panula et al. 1997). Briefly, scores were given for the cartilage structure, cellularity, stainability of the cartilage with safranin O, and the integrity of tidemark.

Synovial fluid (SF) samples

SF specimens were collected immediately after killing the dogs. The hindlimbs were dissected free and the joint capsule of the knee was exposed. 3 mL of sterile 0.9% saline was injected into the suprapatellar pouch. The joint was flexed and extended 10 times and gently massaged to ensure thorough mixing of the saline with the SF. All of the diluted SF was then aspirated from the opened joint and centrifuged ($500 \times g$), after measurement of the collected fluid volume. SF samples were taken from both knees of the dogs. The aliquots were stored frozen at –70 °C pending analysis.

Assays of stromelysin (MMP-3) and tissue inhibitor of metalloproteinases (TIMP-1)

Concentrations of MMP-3 and TIMP-1 were determined by sandwich ELISAs, using monoclonal and polyclonal antibodies, raised against the canine recombinant proteins, as described earlier (Cooksley et al. 1990, Bayne et al. 1992, Lohmander et al. 1993). All assays use a monoclonal antibody against the enzyme or inhibitor as a trapping reagent. Polyclonal antisera against the specific proteins generated in rabbits were used as secondary reagents. The assay for MMP-3 detects the proform of the enzyme, the large molecular active forms of the enzyme and the enzyme complexed to TIMPs, but not small molecular forms of the enzyme or enzyme complexed to alfa-2-macro-

globulin. The assay for TIMP-1, as used in this study, detects only free TIMP-1 (i.e., not the inhibitor complexed with metalloproteinases). The MMP-3 assay has a log-linear range of 0.5–100 ng/mL.

Quantification of chondroitin sulfate (CS)

CS concentration (aggrecan fragments) was determined by precipitation with Alcian Blue, as described by Björnsson (1993).

Phospholipase A_2 (PLA_2) activity assay

The PLA_2 assay method described by Rönkkö et al. (1991) was used. Briefly, PLA_2 activity was measured with 1-acyl-2-[4-pyrenyl]-butyroyl-phosphatidylcholine (PPC) as substrate. The hydrolysis rate of PPC was measured with a fluorescence spectrophotometer (Hitachi 650–50, Tokyo, Japan) and the fluorescence was measured with excitation at 343 nm and emission at 377 nm. All measurements were done in duplicate.

Quantification of hyaluronan (HA)

HA content was measured in duplicate with an ELISA-like method, using a biotinylated HA-binding probe (bHABC) prepared from proteoglycan-link protein complex of bovine articular cartilage (Wang et al. 1992). The assay method used is slightly modified from that described by Kongtawelert and Ghosh (1990). Nunc Covalink plates (Covalink, Nunc, Roskilde, Denmark) were coated with HA (100 µL, 50 µg/mL, Sigma, St. Louis, MO, USA) and blocked with 1% bovine serum albumin (BSA). The samples were first digested for 24 h at 60°C by papain (250 mg/mL in 5 mM cysteine, 5mM EDTA, Sigma); the enzyme then was inactivated by boiling for 10 minutes. The digested samples and a series of standard HA (5–200 ng/mL), diluted in 6% BSA in phosphate-buffered saline (PBS), were mixed with an equal volume of bHABC (450 ng/mL) in small polypropylene tubes (Mekalasi Oy, Kuortane, Finland) and incubated overnight at 4 °C. Immediately before the assay, the mixtures were incubated for 1 h at 37 °C. The samples were then transferred to the HA-coated plates, incubated for 90 min at 37 °C and washed with 0.5% Tween 20 in PBS. Horseradish peroxidase conjugated streptavidin (1:20000 in PBS, Vector Lab, Burlingame, CA, USA) was added to the plates and left for 1 h at 37 °C. After washing, the substrate-chromogen solution (O-phenylenediamine dihydrochloride; Sigma, 0.03% H_2O_2 in 0.1 M citrate buffer, pH 5.0) was added and left for 1 h. The reaction was stopped with 8 M H_2SO_4 and the absorbances were read at 490 nm, using a microtiter plate reader (Molecular Devices Corp, Palo Alto, CA, USA).

Statistics

The Wilcoxon matched-pairs signed-ranks test and the Mann-Whitney U-test were used to assess differences between the operated hindlimb and the contralateral side and between the groups, respectively. For correlations, Pearson's linear correlation analysis was used. A p-value less than 0.05 was considered statistically significant.

Results

Osteotomy

All osteotomies healed well and the mean (SD) tibial angle (i.e., the angle between the axis of the upper metaphysis of the tibia above the osteotomy line and the axis of the tibial shaft) was 29° (5°) and 11° (5°) (group I) and 28° (4°) and 9° (1°) (group II) in the right operated and left control tibia, respectively.

1 month after tibial osteotomy, no limping could be observed, although the forces measured under the paw of the operated hindlimb were about 70% of that on the contralateral side. The difference leveled off 3 months after the operation (Heikkinen et al. 1997).

Cartilage (Tables 1 and 2)

2 of the 7 dogs in group I showed macroscopic fibrillation of cartilage on the medial condyle of the femur in an area 1 mm × 2 mm (Table 1). 5 of 7 valgus-operated dogs in group I had microscopic changes in the cartilage (Table 2). 6 of 9 in the group II valgus-osteotomized animals had macroscopic cartilage lesions in the right knee. 5 of 9 dogs had severe local fibrillation and ulcerative cartilage lesions in the anterior part of the medial condyle of the femur and 4 on the patellar surface of femur. In 3 dogs, the patella cartilage was ulcerated and 2 animals had marginal osteophytes and the normally convex surface of the patella was flattened. 1 dog had cartilage fibrillation on the medial aspect of lateral tibial condyle. 3 of 9 dogs showed no macroscopic changes. In both groups, the contralateral knee joint was normal.

Synovial fluid

In group I animals, the volume of the aspirated rinsing solution was 2.9 mL (SD 0.1) in both knees. In group II, the volume was 3.0 mL (SD 0.1) in the right (operated) knee and 2.9 mL (SD 0.2) in the left (control) knee.

Chondroitin sulfate

The levels (mean (SD), mg/mL) of CS on the operated side were 4.8 (1.9) in group I and 5.7 (2.1) in group II and 4.2 (1.0) and 5.3 (2.1), respectively, in the con-

Table 1. Macroscopic changes in cartilage, bone and synovium in beagle dog stifle joints 7 months (group I) and 18 months (group II) after tibial osteotomy. No changes were observed in the lateral condyle of femur or medial condyle of tibia.

Dog no.	PSF	MF	P	LT	Synovium
Group I: Valgus osteotomy (n 7)					
1	–	Fibrillated cartilage 1 × 2 mm	–	–	–
2	–	Fibrillated cartilage 1 × 2 mm	–	–	–
Group II: Valgus osteotomy (n 9)					
3	Sulcus disappeared	Ulcerated cartilage, whole condyle	Ulcerated flattened surface, medial subluxation, osteophyte in the inferior pole	Fibrillated cartilage 1 × 3 mm	Yellowish, macroscopic synovitis
4	Sulcus flattened, fibrillation	Ulcerated cartilage 4 × 4 mm	–	–	–
5	Sulcus flattened, fibrillation	Ulcerated cartilage 4 × 5 mm and 7 × 5 mm	Ulcerated cartilage 4 × 8 mm, lateral side	–	Yellowish, macroscopic synovitis
6	–	Fibrillated cartilage 5 × 3 mm	–	–	–
7	Sulcus flattened, fibrillation	Ulcerated cartilage 4 × 13 mm	Ulcerated flattened surface, medial subluxation, osteophyte in the inferior pole	–	Yellowish, macroscopic synovitis
8	–	Fibrillated cartilage 5 × 3 mm	–	–	–

Dogs with no changes are not listed. – no changes observed in this joint surface, PSF patellar surface of femur, MF medial condyle of femur, P patella, LT lateral condyle of tibia.

Table 2. Mankin scores of the beagle stifle joints 7 months (group I) and 18 months (group II) after tibial osteotomy. The scores of each animal at different sites of the knee joint are added. The total is the sum of scores

	PSF	MF	LF	P	MT	LT	Total
Group I: Valgus osteotomy (n 7)							
Right knee	5	2	0	2	2	3	14 [a, b]
Left knee	1	1	0	0	0	0	2
Group II: Valgus osteotomy (n 9)							
Right knee	22	14	11	13	8	12	80 [a, b]
Left knee	0	0	0	0	0	0	0

PSF patellar surface of femur, MF medial condyle of femur, LF lateral condyle of femur, P patella, MT medial condyle of tibia, LT lateral condyle of tibia.
[a] $p < 0.05$, between the right and left knee joint in both groups, using the Wilcoxon matched-pairs signed-rank test.
[b] $p < 0.05$, between the right knee in groups I and II, using the Mann-Whitney U-test.

Table 3. Analysis of synovial fluid from the knees of young beagle dogs after valgus osteotomy of the right tibia. Synovial fluid lavage samples were obtained when the animals were killed (group I 7 months, and group II 18 months after operation). Synovial fluid was examined for levels of phospholipase A_2 (PLA_2) activity, tissue inhibitor for metalloproteinases (TIMP) and stromelysin (MMP-3). Mean (SD)

Mediator	Group	Right knee	Left knee
PLA_2 (pmol/min/mL)	I [a]	16.6 (3.2) [c]	14.2 (1.6)
	II [b]	20.1 (7.9)	19.8 (9.7)
TIMP (nM)	I	1.9 (1.6)	1.3 (0.4)
	II	1.1 (0.4) [c]	0.7 (0.2)
MMP-3 (nM)	I	1.2 (0.7) [c]	0.7 (0.2)
	II	2.0 (0.6) [c]	1.3 (0.4)

[a] n 7, group I
[b] n 9, group II
[c] $p < 0.05$ versus left knee joint, using the Wilcoxon matched-pairs signed-ranks test.

tralateral joints. The difference was not statistically significant.

Phospholipase A_2

There was an increase in PLA_2 activity in the operated knee of group I animals, compared to the unoperated side ($p = 0.02$) (Table 3). The difference could not be detected any more in group II animals.

Tissue inhibitor of metalloproteinases

The group II valgus-operated animals showed a rise ($p = 0.04$) in the concentration of TIMP-1 in the operated right knee joints, as compared to the contralateral joints (Table 3).

MMP-3

In both experimental groups, there was an increase in MMP-3 concentration, as compared to the unoperated side ($p = 0.04$) (Table 3).

Molar ratio of MMP-3 to TIMP-1

The ratio (mean MMP-3/mean TIMP-1) in the right knee joint was 0.6 in group I and 1.8 in group II and the left knee joint 0.5 and 1.8, respectively ($p = 0.01$). There was no difference in the molar ratio between the operated and the unoperated joints at either timepoint.

Hyaluronan

The concentration of HA (mean (SD), mg/mL) in the knee joints of the valgus-operated limbs (group I 0.4 (0.3), group II 0.5 (0.2)) did not differ from the contralateral limbs (group I 0.5 (0.3), group II 0.4 (0.2)).

Correlations

In the valgus-operated dogs, the correlation coefficients of SF concentrations of TIMP-1, MMP-3 and the activity of PLA_2, on the one hand, and the severity of cartilage lesions in the same joints, on the other hand, were 0.35, 0.25 and 0.17 in group I and 0.83 ($p = 0.01$), –0.62 and –0.45 in group II, respectively. The correlation coefficients between MMP-3 and TIMP-1 were 0.91 (group I) and –0.21 (group II). These correlations were not significant.

Discussion

Synovial fluid undergoes continuous formation by capillary filtration and removal by transsynovial flow into the lymphatic circulation. The concentration of markers of cartilage is governed by the rate of release of the components into the joint cavity relative to the volumetric rate of SF turnover (Levick et al. 1996). It is conceivable that in different stages of the OA process the rate of release of markers varies, thus influencing the concentration of markers. It is also known that, after a "silent" period of the disease, there are exacerbation phases of OA, further affecting the concentration of markers of cartilage metabolism. As a clinical tool a single measurement of the SF concentration of a marker can be misleading, if the volume of the SF is not considered. The importance of effects of volume and turnover rate of SF on marker concentration has been evaluated by Levick (1990). In our study, we related the concentration of markers to the rinsing solution volume of samples. However, taking into account the small number of animals in this study, we should be careful in interpreting the significance of elevated levels of markers.

We compared the SF marker values of the operated limbs with those of the contralateral knee. The reason why we did not compare the marker levels of the osteotomized dogs and control animals with each other was to avoid the biological variation between individual dogs. Thus, we thought that the contralateral joint would give the most reliable reference values. The paw weight-bearing force was initially diminished in the operated leg, but was at the same level 3 months after the operation and also thereafter no differences could be observed between the operated and contralateral limbs. In the first weeks of increased load-bearing, a change in the contralateral knee joint's articular cartilage metabolism could have taken place, which might have affected the SF marker levels, even at the 7-month interval. However, such a prolonged effect of initial load-bearing seems unlikely. It can also be argued that the elevated load-bearing would bring about negative changes in the contralateral joint cartilage which, in turn, would have tended to reduce, not to enhance, the differences observed between the joint fluids on the operated and contralateral sides.

PLA_2 is a key enzyme in providing substrates for the synthesis of potent mediators of inflammation, including prostaglandins (Pruzanski and Vadas 1991). Inflammatory episodes of varying duration have been noted in human OA (Revell et al. 1988). We found a statistically significant rise in the activity of PLA_2 in joint fluids from valgus-operated knee joints at 7 months, but not at 18 months after operation. PLA_2 originates from the synovial lining cells, inflammatory cells or chondrocytes (Gilman et al. 1988, Gonzales-Buritica et al. 1989, Pruzanski et al. 1990). Increased mechanical stimulation as a signal for initiation of PLA_2 production by the chondrocytes has been suggested by Chrisman and co-workers (1981). It is possible that the tibial valgus angulation stimulated PLA_2 synthesis via a changed mechanical loading. The role of the increased activity of PLA_2 in the initiation of cartilage changes is not clear, but it may be associated with the increased concentrations of MMP-3 in joint fluid.

MMP-3 concentration was increased in SF of the operated limb at both time-points, while the concentration of TIMP-1 was increased only at the later time-point. The increase in the level of TIMP-1 correlated with the severity of cartilage damage. Elevated concentrations of MMP-3 and TIMP-1 have been observed in different types of human arthritides (Lohmander 1991), with molar ratios between total MMP-3 and the free TIMP-1 varying between 1.6 and 5.3 in the different disease groups. The ratio of MMP-3 to TIMP-1 was 0.5 in a healthy human reference group. MMP-3 concentrations in the human disease groups averaged 15–45 times that of the reference group. In our study, the elevation of SF MMP-3 in the valgus-operated joints was much less than in human arthritis. This moderate change is consistent with the small area and the initial phase of degenerated cartilage in the canine knee, as compared to human studies, where the injured cartilage area and the mass of injured cartilage is large. The molar ratio of MMP-3 to its inhibitor TIMP-1 was higher in group II than in group I animals, corresponding to the more severe cartilage damage in group II than in group I (Table 2). It has earlier been suggested that the increased molar ratio

between MMP-3 and TIMP-1 could be due to a changed joint loading pattern or systemic influence (Dahlberg et al. 1994).

The level of synovial fluid CS has been considered to reflect the metabolism of aggrecan. The level of aggrecan fragments in SF increases during the early course of OA and RA (Witter et al. 1987, Lohmander et al. 1993). SF CS concentrations did not differ between the operated and nonoperated knees, although the level of CS tended to be higher in the operated hindlimb than on the contralateral side. These results in dogs contrast with previous human data showing an inverse relation between the radiographic severity of knee OA and the concentration of joint fluid proteoglycan fragments (Dahlberg et al. 1992). A likely explanation of this discrepancy is that in our dog model, cartilage changes were at a very early stage and the damaged joint area was rather limited. It is also known that in early-stage cartilage lesions both the synthesis and degradation of aggrecan are activated (Carney et al. 1984, Adams and Brandt 1991).

The SF HA concentration in the knee of valgus-operated dogs did not differ from the control joint values. This is in contrast to results in dogs with advanced secondary OA, which show significantly decreased SF HA concentrations in the affected joints as compared with the unaffected contralateral joints (Arican et al. 1994). The reason for this observation is probably the same as discussed above.

In summary, the changes observed in the concentrations of synovial fluid markers in this slowly progressive model of OA, after tibial valgus osteotomy, suggest the activation of inflammation-related processes in synovium, cartilage or other joint tissues already at a very early stage of the disease. The increased concentration of MMP-3 in joint fluid is consistent with the increased activity of PLA_2, and probably reflects the increased rate of matrix cartilage metabolism in early OA.

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References

Adams M E, Billingham M E J. Animal models of degenerative joint disease. Curr Top Pathol 1984; 7: 265-97.

Adams M E, Brandt K D. Hypertrophic repair of canine articular cartilage in osteoarthritis after anterior cruciate ligament transection. J Rheumatol 1991; 18: 428-35.

Arican M, Carter S D, May C, Bennett D. Hyaluronan in canine arthropathies. J Comp Path 1994; 111: 185-95.

Bayne E K, Hutchinson N I, Walakovits L A, Donatelli S, MacNaul K L, Harper C F, Cameron P, Moore V L, Lark M W. Production, purification and characterization of canine prostromelysin. Matrix 1992; 12: 173-84.

Björnsson S. Simultaneous preparation and quantification of proteoglycans by precipitation with Alcian Blue. Anal Biochem 1993; 210: 282-91.

Carney S L, Billingham M E J, Muir H, Sandy J D. Demonstration of increased proteoglycan turnover in cartilage explants from dogs with experimental osteoarthritis. J Orthop Res 1984; 2: 201-6.

Chrisman O D, Ladenbauer-Bellis J M, Panjabj M. The relationship of mechanical trauma and the early biomechanical reactions of osteoarthritic cartilage. Clin Orthop 1981; 161: 275-84.

Clark I M, Powell L K, Ramsey S, Hazleman B L, Cawston T E. The measurement of collagenase, tissue inhibitor of metalloproteinases (TIMP), and collagenase-TIMP complex in synovial fluids from patients with osteoarthritis and rheumatoid arthritis. Arthritis Rheum 1993; 36: 372-9.

Cooksley S, Hipkiss J B, Tickle S P, Holmesievers E, Docherty A J P, Murphy G, Lawson A D G. Immunoassays for the detection of human collagenase; stromelysin; tissue inhibitor of metalloproteinases (TIMP-1) and enzyme-inhibitor complexes. Matrix 1990; 10: 285-91.

Dahl L B, Dahl I M, Engström-Laurent A, Granath K. Concentration and molecular weight of sodium hyaluronate in synovial fluid from patients with rheumatoid arthritis and other arthropathies. Ann Rheum Dis 1985; 44: 817-22.

Dahlberg L, Ryd L, Heinegård D, Lohmander L S. Proteoglycan fragments in joint fluid - Influence of arthrosis and inflammation. Acta Orthop Scand 1992; 4: 413-6.

Dahlberg L, Roos H, Saxne T, Heinegård D, Lark M L, Hoerrner L A, Lohmander L S. Cartilage metabolism in the injured and uninjured knee of the same patient. Analysis of aggrecan fragments, cartilage matrix oligomeric protein, stromelysin, and TIMP in synovial fluid, and COMP in serum. Ann Rheum Dis 1994; 53: 823-7.

Dean D D, Martel-Pelletier J, Pelletier J-P, Howell D S, Woessner J F Jr. Evidence for metalloproteinase and metalloproteinase inhibitor imbalance in human osteoarthritic cartilage. J Clin Invest 1989; 84: 678-85.

Dingle J T, Saklatvala J, Hembry R, Tyler J, Fell H B, Jubb R. A cartilage catabolic factor from synovium. Biochem J 1979; 184: 177-80.

Gilman S C, Chang J, Zeigler P R, Uhl J, Mochan E. Interleukin-1 activates phospholipase A2 in human synovial cells. Arthritis Rheum 1988; 31: 126-30.

Gonzales-Buritica H, Smith D M, Turner R A. Characterization of soluble and cell-associated phospholipase A2 from rheumatoid synovial fluid. Ann Rheum Dis 1989; 48: 557-64.

Heikkinen L M, Panula H E, Lyyra T, Olkkonen H, Kiviranta I, Nevalainen T, Helminen H J. Electromechanical film sensor device for dynamic force recordings from canine limbs. Scand J Lab Anim Sci 1997; 24: 85-92.

Johnson R G, Poole A R. Degenerative changes in dog articular cartilage induced by a unilateral tibial valgus osteotomy. Exp Pathol 1988; 33: 145-64.

Kongtawelert P, Ghosh P. A method for the quantification of hyaluronan (hyaluronic acid) in biological fluids using a labelled avidin-biotin technique. Anal Biochem 1990; 185: 313-8.

Kortekangas P, Aro H T, Nevalainen T J. Group II phospholipase A2 in synovial fluid and serum in acute arthritis. Scand J Rheumatol 1994; 2: 68-72.

Levick J R. The "clearance" of macromolecular substances such as cartilage markers from synovial fluid and serum. In: Methods in cartilage research (Eds. Maroudas E, Kuettner K). Academic Press. London 1990; 352-357.

Levick J R, Price F M, Mason R M. Synovial matrix-synovial fluid system of joints. In: Extracellular matrix (Ed. Comper W D). Harwood Academic Publishers GmbH. Amsterdam 1996 1: 328-77.

Lohmander L S. Markers of cartilage metabolism in arthrosis. A review. Acta Orthop Scand 1991; 62: 623-32.

Lohmander L S, Hoerrner L A, Lark M W. Metalloproteinases, tissue inhibitor, and proteoglycan fragments in knee synovial fluid in human osteoarthritis. Arthritis Rheum 1993; 2: 181-9.

Murphy G. Matrix metalloproteinases and their inhibitors. Acta Orthop Scand (Suppl 266) 1995; 66: 51-4.

Myers S L, Brandt K D, O'Connor B L, Visco D M, Albrecht M E. Synovitis and osteoarthritic changes in canine articular cartilage after anterior cruciate ligament transection: Effects of surgical hemostasis. Arthritis Rheum 1990; 33: 1406-15.

Ogston A G, Steiner J E. The physiological function of hyaluronic acid in synovial fluid: viscous, elastic and lubricant properties. J Physiol 1953; 119: 244-52.

Panula H E, Helminen H J, Kiviranta I. Slowly progressive osteoarthritis after tibial valgus osteotomy in young beagle dogs. Clin Orthop 1997; in press.

Pelletier J-P, Martel-Pelletier J, Malemud C J. Canine osteoarthritis: effects of endogenous neutral metalloproteoglycanases on articular cartilage proteoglycans. J Orthop Res 1988; 6: 379-88.

Pond M J, Nuki G. Experimentally-induced osteoarthrosis in the dog. Ann Rheum Dis 1973; 32: 387-8.

Pruzanski W, Bogoch E, Stefanski E, Wloch M, Vadas P. Synthesis and release of phospholipase A2 by unstimulated human articular chondrocytes. J Rheumatol 1990; 17: 1386-91.

Pruzanski W, Vadas P. Phospholipase A2 - a mediator between proximal and distal effectors of inflammation. Immunol Today 1991; 12:143-6.

Reimann I. Experimental osteoarthrosis of the knee in rabbits induced by alteration of the load-bearing. Acta Orthop Scand 1973; 44: 496-504.

Revell P A, Mayston V, Lalor P, Mapp P. The synovial membrane in osteoarthritis: a histological study including the characterization of the cellular infiltrate present in inflammatory osteoarthritis using monoclonal antibodies. Ann Rheum Dis 1988; 47: 300-7.

Rönkkö S, Lahtinen R, Vanha-Perttula T. Phospholipases A_2 in the reproductive system of the bull. Int J Biochem 1991; 23: 595-603.

Sandy J D, Flannery C R, Neame P J, Lohmander L S. The structure of aggrecan fragments in human synovial fluid: Evidence for the involvement in osteoarthritis of a novel proteinase which cleaves the glu 373-ala 374 bond of the interclobular domain. J Clin Invest 1992; 89: 1512-6.

Vadas P, Stefanski E, Pruzanski W. Characterization of extracellular phospholipase A2 in rheumatoid synovial fluid. Life Sci 1985; 36: 579-87.

Wang C, Tammi M, Tammi R. Distribution of HA and its CD44 receptor in the epithelia of human skin appendages. Histochemistry 1992; 98: 105-12.

Witter J, Roughley P J, Webber C, Roberts N, Keystone E, Poole A R. The immunologic detection and characterization of cartilage proteoglycan degradation products in synovial fluids of patients with arthritis. Arthritis Rheum 1987; 30: 519-29.

Wu J J, Lark M W, Chun L E, Eyre Dr. Sites of stromelysin cleavage in collagen types II, IX, X and XI of cartilage. J Biol Chem 1991; 266: 5625-8.