J Vet Clin 26(2) : 144-149 (2009)

Journal of Veterinary Clinics
www.ksvc.or.kr

Proteinases and their Inhibitors in Cartilage and Synovial Fluid Acquired from a Canine Osteoarthritic Model

Jae-Won Seol, Hae-Beom Lee, Nam-Soo Kim, Young-Hoon Lee*, Hyung-Sub Kang, In-Shik Kim and Sang-Youel Park[1]

Center for Healthcare Technology Development, Bio-Safety Research Institute, College of Veterinary Medicine, Chonbuk National University, Jeonju 561-756, Korea
**Institute of Oral Bioscience, School of Dentistry, Chonbuk National University, Jeonju 561-756, Korea*

(Accepted: April 06, 2009)

Abstract : Chondrocytes and synovial fluid derived markers are used to monitor for osteoarthritis (OA). Specific inhibitors, known as tissue inhibitors of metalloproteinases (TIMP), regulate the proteolytic activity of matrix metalloproteinases (MMP). This study investigated whether MMP and TIMP levels were altered in synovial fluid and cartilage following the experimental induction of OA in canines. Twenty mature beagle dogs underwent a unilateral surgical transection of the cranial cruciate ligament and the medial collateral ligament as well as a medial meniscectomy. Matrix metalloproteinase-2 and MMP-9 levels were assayed using Western blot and TIMP-2 levels were measured with enzyme-linked immunosorbent assays four weeks after OA induction. Increased MMP-2 expression was observed in chondrocytes isolated from cartilage following OA induction, but MMP-9 expression decreased. Matrix metalloproteinase-2 and MMP-9 levels in synovial fluid from the OA induced joint significantly increased compared to those of the sham group. Tissue inhibitors of metalloproteinase-2 concentrations were higher in chondrocytes from the OA cartilage, yet TIMP-2 remained lower in the synovial fluid of OA. This suggests the elevated release of MMP-9 over MMP-2 into the synovial fluid following the cartilage degradation-related death of chondrocytes after OA. Osteoarthritis can be further deteriorated by increased MMP activity in the synovial fluid because TIMP-2 exist low concentration into the extracellular matrix. As a result, MMP activity, particularly MMP-9 activity, can be useful as a biomarker in diagnosing and monitoring the early stages of canine OA.

Key words **:** MMP, TIMP, osteoarthritis, cartilage, synovial fluid.

Introduction

Osteoarthritis (OA) is a syndrome caused by the convergence of different etiologies on the same pathogenic pathway, resulting in the production of characteristic changes in cartilage, subchondral bone, and the synovial membrane. As a result of cartilage breakdown and its metabolic modifications, several molecules appear in differing amounts in the synovial fluid of the affected joints (15). The diagnosis of OA is based upon clinical orthopedic examination and radiographic assessments, both of which can be vague and impervious to early joint disease. Recent studies have included magnetic resonance imaging (MRI), computed tomography (CT), and the evaluation of biochemical markers within the synovial fluid (e.g. metalloproteinases, inhibitors of metalloproteinases, keratin sulphate epitopes, nitric oxide, chondroitin sulphate, osteocalcin, hyaluronan, antibodies to collagen I and II, and others) (2-4,9,10,23).

Matrix metalloproteinases (MMP) refer to a group of Zn^{2+} dependent extracellular enzymes that play a key role in normal and pathological tissue remodeling and have the combined ability to degrade all components of the extracellular matrix (ECM) (13,17,18). The MMP family of proteinase includes collagenases 1 and 3 (MMP-1 and MMP-3), gelatinases (MMP-2 and MMP-9), and stromelysis (MMP-3). MMPs are essential for the normal development and turnover of the articular cartilage extracellular matrix (10,19). However, their elevated activity in osteoarthritis and rheumatoid arthritis can lead to the excessive destruction of the ECM. Tissue inhibitors of metalloproteinase (TIMP), of which TIMP-1 And TIMP-2 are most important, naturally regulate MMP. Tissue inhibitors of metalloproteinase bind to the active site on the MMP enzyme to form a 1:1 non-covalent complex that inhibits MMP activity. Imbalances in MMP and TIMP levels have been determined in cartilage from OA patients (6). Therefore, MMPs and TIMPs are generally believed to be effective OA biomarkers.

In the present study, the canine OA model was used to study biochemical factors involving MMPs and TIMPs, and to conduct a unilateral surgical transection of the cranial cruciate ligament, the medial collateral ligament, and a medial meniscectomy. The study also investigated whether MMP

[1]Corresponding author.
E-mail : sypark@chonbuk.ac.kr

and TIMP expression was altered in synovial fluid and chondrocytes from the experimentally induced canine OA model.

Materials and Methods

Osteoarthritis induction

Twenty beagle dogs, 1-2 years of age (mean: 1.4±0.4 years) with a weight of 9-11 kg (mean: 10.2±1.4 kg), were used. The dogs were given intravenous crystalloid fluids (10 mL/kg/hr). Anesthesia was induced with 6 mg/kg of propofol (Anepofol Inj®, Hana Pharm. Co. Ltd.; Korea) and maintained with enflurane under 100% oxygen. The cranial cruciate ligament and the medial collateral ligament were transected, and a medial meniscectomy was carried out according to standard procedures (20). The stifle joint medial arthrotomy was carried out for sham groups.

Macroscopy and histochemical examination of the articular cartilage

The experimental dogs were sacrificed at 12 weeks to evaluate the severity of OA following surgery. The level of macroscopic synovial inflammation and cartilage damage were evaluated on digital high-resolution photographs. Histochemical staining was performed on frozen 15 micrometer sections of the articular cartilage four weeks following OA induction. All reagents for histochemical staining were obtained from a commercial supplier (Sigma Chemical Co.; St. Louis, MO, USA). A solution of naphthol AS-BI phosphate in 2.5 mL of n-dimethyformamide to which 40 mL of 0.05 M Tris-malate buffer (pH 5) was then added. To prepare a solution of hexazotized pararosaniline, 80 mg of pararosaniline hydrochloride was dissolved in 1.6 mL of distilled water and 0.4 mL of hydrochloric acid was then added. This solution was mixed with an equal volume of 4% sodium nitrite immediately before use. The final reaction mixture for the histochemical staining was prepared by adding 4 mL of the hexazotized pararosaniline solution to the naphthol AS=BI phosphate solution, along with 50 mM of sodium-potassium tartrate. The final reaction mixture (pH 5) was filtered prior to use. The cartilage were incubated in the reaction mixture at 37°C for 1 hour, rinsed in distilled water, counterstained with Mayer hematoxylin, and then mounted. For each batch of slides, a negative control was prepared by omiting naphthol AS-BI phosphate. The articular cartilage was examined using light microscopy.

Preparation of synovial fluid

The synovial fluid was collected four weeks after OA induction in all experimentally dogs. Briefly, the dogs were sedated with a 0.2 mg/kg acepromazine (Sedazect Inj®, Samwoo Pharm. Co. Ltd.; Korea) IV and placed in ventrodorsal recumbency with the stifle joint flexed. Digital pressure was applied to the medial side of the straight patellar ligament and a 21-gauge spine needle was inserted with a 5 mL plastic syringe through the tat pad toward the intercondylar space lateral to the straight patellar ligament. Available synovial fluid was collected and care was taken to prevent contamination with blood. The synovial fluid was measured and centrifuged at 4°C for 10 minutes at 12,000 rpm. The supernatant were stored at −80°C until assay.

Western blot assay

Chondrocytes were isolated from cartilage obtained from the osteoarthritis joints. The cells were collected by centrifugation, washed twice with a phosphate buffer saline (PBS), and resuspended in a lysis buffer [25 mM HEPES (pH 7.4), 100 mM NaCl, 1 mM ethylene diamine tetraacetic acid (EDTA), 5 mM $MgCl_2$, 0.1 mM dithiothreitol (DTT), and protease inhibitor mixture]. Synovial fluids were diluted 10 times using phosphate buffer and a protein assay was performed using Western blotting. Equal amounts of the lysate protein were run on 8-15% SDS-polyacrylamide gels and electrophoretically transferred to nitrocellulose membrane. The nitrocellulose blots were blocked with 5% nonfat dry milk in the PBS with a Tween 20 buffer and incubated with the primary antibody. Immunoreactivity was detected by sequential incubation with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (ECL) reagents. The MMP-2 and MMP–9 antibodies used for Western blotting analysis were obtained from CHEMICON (USA). The intensities of the bands obtained after Western blot analysis were measured by laser scanning densitometry (Bio-Rad Laboratories Inc.; Hercules, CA, USA) and analyzed as a percentage (%) compared to the sham group.

TIMP-2 assay

Existing TIMP-2 was measured in the synovial fluid and chondrocytes removed from the experimentally induced OA joints and complexed with active forms of MMP using a commercially available TIMP-2 Biotrack assay kit (Amersham Bioscience Inc., Piscataway, NJ, USA). Synovial fluid and lysate protein from the chondrocytes were briefly diluted 1:4 in a 0.03 M phosphate assay buffer containing 0.1 M sodium chloride. 100 μl mixed samples were placed in the appropriate well and incubated at 20-27°C for 2 hours. Subsequently, all wells were aspirated and washed four times with a 0.01 M phosphate buffer (pH 7.5) containing 0.05 M sodium chloride and 0.05% Tween. One-hundred μl of the equilibrated teramethylbenzidine (TMB) substrate was placed into each well, and the plate was covered and left to stand for 30 min at 20-27°C. One-hundred μl of 1 M sulfuric acid was added to the wells to extinguish the reaction, and the plate was read at a wavelength of 450 nm within 30 min. The TIMP-2 concentrations were determined from a standard curve and are reported in ng/mL.

Statistical analysis

All data are expressed as the mean±standard deviation (SD), and the data was compared using the Student's T-test

and the ANOVAs Duncan test with the SAS statistical package. The results were considered significant at a value of *P < 0.05, **P < 0.01.

Results

Macroscopic and histochemical examination of the articular cartilage

To evaluate macroscopic damage to the articular cartilage as a result of OA induction, cartilage from the stifle joint was observed. Damage to the cartilage was found on the tibial plateau of the experimental joints. The cartilage of the medial tibia plateau was fibrinoid and demonstrated erosion (Fig 1A). Under microscope, the cartilage of the experimental joint was hypertrophied, as characterized by scattered chondrocytes (Fig 1B).

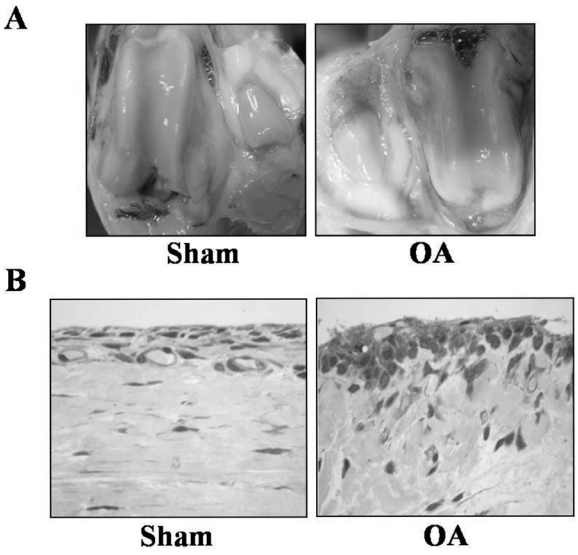

Fig 1. Evaluation of articular cartilage following experimentally induced osteoarthritis. (A) Photomicrograph of the articular cartilage. (B) Histochemical stains were performed on frozen 15 μm sections of articular cartilage four weeks following the OA induction. OA; Osteoarthritis.

Matrix metalloproteinase-2 and MMP-9 measurements in cartilage and synovial fluid acquired from experimentally induced OA

Specific inhibitors known as tissue inhibitors of metalloproteinase (TIMP-2) regulate the proteolytic activity of MMP. The present study investigated whether MMP and TIMP levels were altered in synovial fluid and cartilage following experimentally induced OA. Matrix metalloproteinase-2 and MMP-9 levels were assayed in chondrocytes isolated from cartilage four weeks following OA induction. Compared to the sham group, MMP-2 levels in experimental cartilage increased, and MMP-9 levels decreased (Fig 2A). To further confirm these results, we carried out a densitometric analysis of MMP-2 and MMP-9 levels (Fig 2B). Percentage variations of the OA cartilage compared with sham cartilage (100%) were 151.7±14.3 (MMP-2) and 63.8±18.4 (MMP-9).

In addition, we examined MMP-2 and MMP-9 levels in synovial fluid originating from the OA induced joint using Western blot. Matrix metalloproteinase-2 and MMP-9 levels in the OA synovial fluid were significantly elevated compared to the sham group (Fig 3A). The densitometric values of MMP-2 and MMP-9 proteins in the synovial fluid were 140.2±12.4 and 129.8±10.2, respectively (Fig 3B).

Measurement of tissue inhibitor of matrix metalloproteinase-2 (TIMP-2) levels in cartilage and synovial fluid originating from experimentally induced OA

The tissue inhibitor of matrix metalloproteinase-2 levels in the synovial fluid and chondrocytes were measured with enzyme-linked immunosorbent assays four weeks following

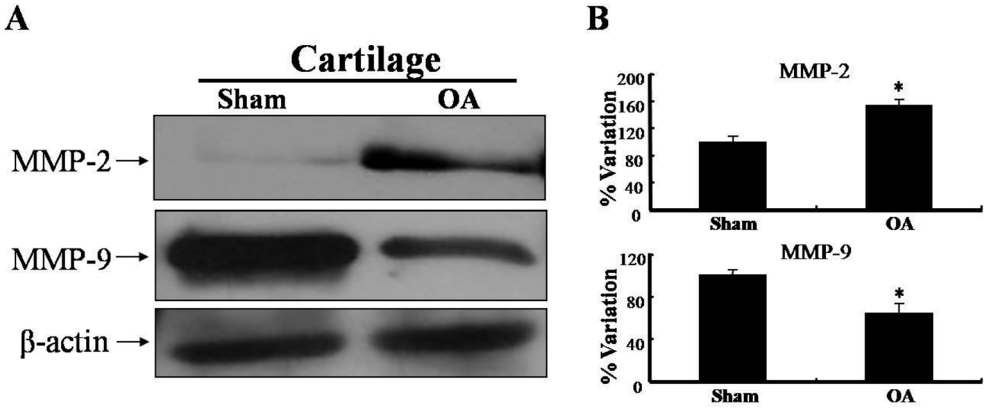

Fig 2. The expression of MMP-2 and MMP-9 was altered in cartilage at the OA induction joint. (A) Chondrocytes were isolated from cartilage obtained from the osteoarthritis joints. β-actin indicates a non-specific protein band used to ensure equal protein loading. (B) Western blotting results were analyzed with image analysis software. Data are expressed as the percentage of sham group protein expression. Standard error is expressed as $p < 0.05$ according to the student's *t*-test. Images of gels represent results for one of the three independent experiments. OA; Osteoarthritis.

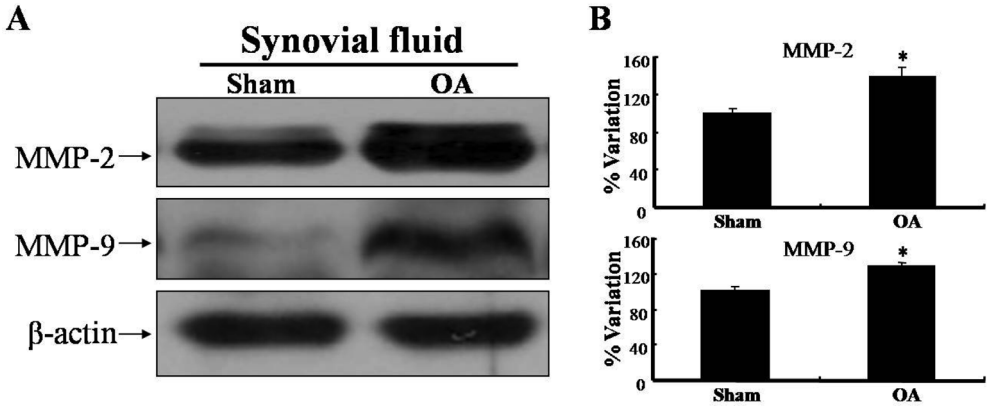

Fig 3. The expression of MMP-2 and MMP-9 increased in the synovial fluid at the OA induction joint. (A) The synovial fluid was collected four weeks following OA induction. β-actin indicates a non-specific protein band used to ensure equal protein loading. (B) Western blotting results were analyzed with image analysis software. Data are expressed as the percentage of sham group protein expression. The bar indicates standard error. *$p < 0.05$ vs. sham sample; Student's *t*-test. Images of gels represent the results for one of the three independent experiments. OA; Osteoarthritis.

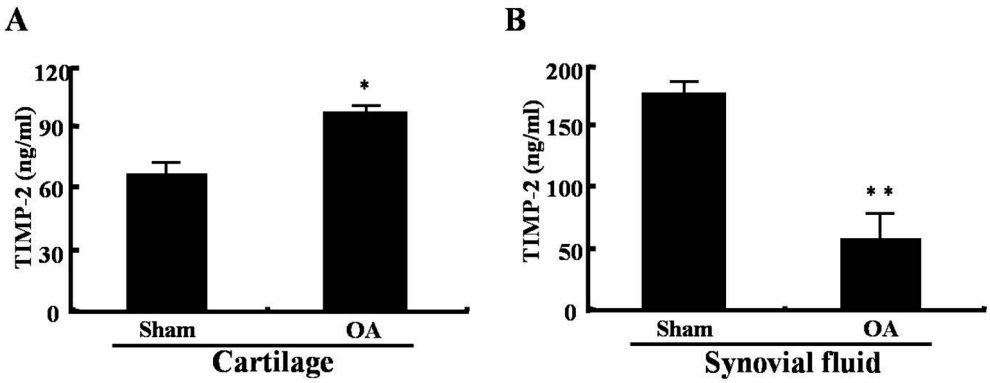

Fig 4. Tissue inhibitor of matrix metalloproteinase-2 concentrations in the experimental synovial fluid were significantly lower than the sham group. (A) The TIMP-2 measurements are taken using the TIMP-2 Biotrack assay kit for chondrocytes acquired from the experimental OA cartilage. Cell lysates were diluted 1:4 in an assay buffer and absorbance values were determined at a wavelength of 450 nm. (B) The TIMP-2 concentrations were measured in the synovial fluid. The bar indicates standard error. *$p < 0.05$, **$p < 0.01$, sham vs. OA (t-test). OA; Osteoarthritis.

OA induction. In the OA cartilage chondrocytes, TIMP-2 levels were higher than values obtained for the sham cartilage (Fig 4A). Following OA induction, TIMP-2 concentration in the chondrocytes was 98.7±7.3 ng/mL. The normal chondrocytes, on the other hand, had a TIMP-2 concentration of 66.3±4.4 ng/mL. In addition, we measured TIMP-2 concentrations in synovial fluid originating from the OA induced joint. These TIMP-2 levels were significantly lower than values obtained from the sham group (Fig 4B). Following induction, the synovial fluid TIMP-2 concentration was 57.2±18.2 ng/mL. Synovial fluid from the sham group had a concentration of 177.2±8.6 ng/mL.

Discussion

Arthritis is a disorder that causes pain, stiffness, and swelling in or around the joints. The two common types of arthritis include rheumatoid and osteoarthritis. Osteoarthritis (OA) is the most common cartilage and joint disease related to advancing age and is characterized by the destruction of articular cartilage. However, other tissues, including bone (7), synovium (11), fat, and ligaments (16,22), are also altered in OA joints and may be involved in the pathogenesis of the disease. The etiology of OA varies among individuals, with possible contributions from genetics and obesity, as well as local biomechanical factors, such as muscle weakness, joint laxity, and traumatic injury.

Up to 17.8% of dogs in purebred populations demonstrate radiographic evidence of elbow OA (25), a condition that often supercedes other canine medical conditions including a fragmented medial coronoid process (FCP). The assessment of canine OA can be achieved through a number of invasive

and non-invasive means including clinical examination, radiography, computed tomography, magnetic resonance imaging, gross examination, histological evaluation, and molecular measures. In the present OA model, the fibrillation of the articular surface as well as chondrocyte death with clone formation in tibial plateau was observed using histological analysis. Histological results obtained in this OA model were similar to previous cranial cruciate ligament transection models conducted with postoperative exercise (14,20).

Significant work has been conducted in previous years to determine the markers of articular cartilage metabolism. Molecules regarded as promising markers include matrix metalloproteinase (MMP) and the tissue inhibitor of matrix metalloproteinase (TIMP), which are thought to play a significant role in the cartilage damage process (8,12,21). In particular, MMP-2 and MMP-9 have three repeats of fibronectin-type domain inserted in the catalytic domain that interact with collagens and gelatins (1,24) and are significantly involved in the initiation and progression of the disease. In this study, we measured MMP-2 and MMP-9 levels in chondrocytes and synovial fluid following the experimental induction of OA. Matrix metalloproteinase-2 expression in the cartilage increased following OA induction, but MMP-9 expression significantly decreased compared to the sham group. This suggests that MMP-9 is released in greater quantities into the extracellular environment than MMP-2 following the cartilage degradation-related death of chondrocytes in OA. Matrix metallproteinase-2 and MMP-9 levels in the synovial fluid originating from OA induced joints were also significantly elevated compared to those of the sham group. This indicates that MMP flows into the synovial fluid as a result of damage to articular cartilage and chondrocytes in OA.

MMPs are dependent on the activation of pro-enzymes and are regulated by specific inhibitors such as α_2-macroglobulin and TIMPs (26). Four TIMPs (TIMP-1, TIMP-2, TIMP-3, and TIMP-4) have been identified in vertebrates (5), and their expression is regulated during development and tissue remodeling. Disruption of the balance between these proteinases and their inhibitors can lead to proteolysis in the inflamed joints (12,27). The present study investigated whether TIMP-2 levels were altered in the synovial fluid and chondrocytes following the induction of OA. Our results demonstrate that TIMP-2 concentrations were higher in chondrocytes from the OA cartilage, but lower in the synovial fluid. These data suggest that TIMP-2 exist as low concentration into the extracellular matrix, resulting in further deteriorated osteoarthritis. This effect is exacerbated by increased MMP activity in the extracellular environment.

Conclusion

This study examined whether MMP and TIMP expression was altered in the articular chondrocytes and synovial fluid following the experimental induction of OA in canines. Matrix metalloproteinase-2 expression in chondrocytes isolated from OA cartilage increased, whereas MMP-9 expression decreased. However, MMP-2 and MMP-9 levels in the synovial fluid from OA induced joints were significantly elevated compared to those of the sham group. Our results also demonstrated that TIMP-2 concentrations were higher in OA cartilage chondrocytes, but lower in OA synovial fluid. This suggests the elevated release of MMP-9 over MMP-2 into the synovial fluid following the cartilage degradation-related death of chondrocytes following OA. MMP activity, particularly MMP-9, can be use as a biomarker for diagnosing and monitoring the early stages of OA. Furthermore, TIMP-2 in post-OA chondrocytes was not released into the extracellular matrix, indicating that osteoarthritis can experience greater deterioration as a result of increased MMP activity in the synovial fluid.

Acknowledgements

This work was supported by a Grant of the Korean Ministry of Education, Science, and Technology (The Regional Core Research Program/Center for Healthcare Technology Development).

References

1. Allan JA, Docherty AJ, Barker PJ, Huskisson NS, Reynolds JJ, Murphy G. Binding of gelatinases A and B to type-I collagen and other matrix components. Biochem J 1995; 309 (Pt 1): 299-306.
2. Arican M, Carter SD, Bennett D, May C. Measurement of glycosaminoglycans and keratan sulphate in canine arthropathies. Res Vet Sci 1994; 56: 290-297.
3. Arican M, Carter SD, Bennett D. Osteocalcin in canine joint diseases. Br Vet J 1996; 152: 411-423.
4. Arican M, Carter SD, Bennett D, Ross G, Ayad S. Increased metabolism of collagen VI in canine osteoarthritis. J Comp Pathol 1996; 114: 249-256.
5. Brew K, Dinakarpandian D, Nagase H. Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biophys Acta 2000; 1477: 267-283.
6. Dean DD, Martel-Pelletier J, Pelletier JP, Howell DS, Woessner JF, Jr. Evidence for metalloproteinase and metalloproteinase inhibitor imbalance in human osteoarthritic cartilage. J Clin Invest 1989; 84: 678-685.
7. Dingle JT. Catabolin--a cartilage catabolic factor from synovium. Clin Orthop Relat Res 1981; 219-231.
8. Green MJ, Gough AK, Devlin J, Smith J, Astin P, Taylor D, Emery P. Serum MMP-3 and MMP-1 and progression of joint damage in early rheumatoid arthritis. Rheumatology (Oxford) 2003; 42: 83-88.
9. Hegemann N, Kohn B, Brunnberg L, Schmidt MF. Biomarkers of joint tissue metabolism in canine osteoarthritic and arthritic joint disorders. Osteoarthritis Cartilage 2002; 10: 714-721.
10. Hegemann N, Wondimu A, Ullrich K, Schmidt MF. Synovial MMP-3 and TIMP-1 levels and their correlation with cytokine expression in canine rheumatoid arthritis. Vet

Immunol Immunopathol 2003; 91: 199-204.
11. Hill CL, Seo GS, Gale D, Totterman S, Gale ME, Felson DT. Cruciate ligament integrity in osteoarthritis of the knee. Arthritis Rheum 2005; 52: 794-799.
12. Ishiguro N, Ito T, Obata K, Fujimoto N, Iwata H. Determination of stromelysin-1, 72 and 92 kDa type IV collagenase, tissue inhibitor of metalloproteinase-1 (TIMP-1), and TIMP-2 in synovial fluid and serum from patients with rheumatoid arthritis. J Rheumatol 1996; 23: 1599-1604.
13. Knauper V, Bailey L, Worley JR, Soloway P, Patterson ML, Murphy G. Cellular activation of proMMP-13 by MT1-MMP depends on the C-terminal domain of MMP-13. FEBS Lett 2002; 532: 127-130.
14. Little C, Smith S, Ghosh P, Bellenger C. Histomorphological and immunohistochemical evaluation of joint changes in a model of osteoarthritis induced by lateral meniscectomy in sheep. J Rheumatol 1997; 24: 2199-2209.
15. Lohmander LS. What is the current status of biochemical markers in the diagnosis, prognosis and monitoring of osteoarthritis? Baillieres Clin Rheumatol 1997; 11: 711-726.
16. Morgan JP, Wind A, Davidson AP. Bone dysplasias in the labrador retriever: a radiographic study. J Am Anim Hosp Assoc 1999; 35: 332-340.
17. Murphy G, Knauper V, Atkinson S, Butler G, English W, Hutton M, Stracke J, Clark I. Matrix metalloproteinases in arthritic disease. Arthritis Res 2002; 4 Suppl 3: S39-49.
18. Nagase H, Woessner JF, Jr. Matrix metalloproteinases. J Biol Chem 1999; 274: 21491-21494.
19. Panula HE, Lohmander LS, Ronkko S, Agren U, Helminen HJ, Kiviranta I. Elevated levels of synovial fluid PLA2, stromelysin (MMP-3) and TIMP in early osteoarthrosis after tibial valgus osteotomy in young beagle dogs. Acta Orthop Scand 1998; 69: 152-158.
20. Pond MJ, Nuki G. Experimentally-induced osteoarthritis in the dog. Ann Rheum Dis 1973; 32: 387-388.
21. Posthumus MD, Limburg PC, Westra J, van Leeuwen MA, van Rijswijk MH. Serum matrix metalloproteinase 3 levels in comparison to C-reactive protein in periods with and without progression of radiological damage in patients with early rheumatoid arthritis. Clin Exp Rheumatol 2003; 21: 465-472.
22. Rogers J, Shepstone L, Dieppe P. Is osteoarthritis a systemic disorder of bone? Arthritis Rheum 2004; 50: 452-457.
23. Spreng D, Sigrist N, Schweighauser A, Busato A, Schawalder P. Endogenous nitric oxide production in canine osteoarthritis: Detection in urine, serum, and synovial fluid specimens. Vet Surg 2001; 30: 191-199.
24. Steffensen B, Wallon UM, Overall CM. Extracellular matrix binding properties of recombinant fibronectin type II-like modules of human 72-kDa gelatinase/type IV collagenase. High affinity binding to native type I collagen but not native type IV collagen. J Biol Chem 1995; 270: 11555-11566.
25. Ushiyama T, Chano T, Inoue K, Matsusue Y. Cytokine production in the infrapatellar fat pad: another source of cytokines in knee synovial fluids. Ann Rheum Dis 2003; 62: 108-112.
26. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 2003; 92: 827-839.
27. Yoshihara Y, Nakamura H, Obata K, Yamada H, Hayakawa T, Fujikawa K, Okada Y. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis. Ann Rheum Dis 2000; 59: 455-461.

개 퇴행성 관절염 모델을 이용한 연골과 활액 내 단백질 분해 효소와 억제제의 작용 연구

설재원 · 이해범 · 김남수 · 이영훈* · 강형섭 · 김인식 · 박상열[1]

헬스케어 기술개발 사업단, 전북대학교 수의과대학 생체안전성 연구소, *전북대학교 치과대학 구강생체과학연구소

요 약 : 퇴행성관절염(Osteoarthritis,OA)은 관절 부위의 퇴행성 변화가 특징이며, 이를 진단하기 위해서는 연골세포나 활액에서 유래된 표지인자가 일반적으로 사용된다. 이번 연구에서는 개를 이용하여 실험적으로 퇴행성관절염을 유도하고, 활액과 연골세포에서 단백질 분해 효소인 matrix metalloproteinase (MMPs)와 MMPs의 활성을 억제시키는 것으로 알려진 tissue inhibitors of metalloproteinases (TIMPs)의 발현 정도를 조사하였다. 20마리의 비글견이 퇴행성관절염 모델로 사용되었으며 MMP-2 와 -9은 Western blot 분석에 의해서, TIMP-2의 농도는 ELISA (enzyme-linked immunosorbent assays)에 의해 결정하였다. 퇴행성관절염 유도 4주 후에 연골에서 분리한 연골세포에서 MMP-2의 발현은 증가되었지만 MMP-9의 발현은 감소되었다. 그러나, 퇴행성관절염을 유도한 개의 활액에서는 MMP-2와 -9의 발현이 모두 증가하는 것을 보였다. TIMP-2의 농도는 퇴행성관절염을 유도한 연골에서 분리한 연골세포에서는 높았지만, 활액에서는 낮은 농도를 보였다. 이러한 결과는 MMP-9가 퇴행성관절염 시 연골 조직의 변성에 따른 연골세포의 손상에 의해 MMP-2보다 더 활액으로 방출된다는 것을 보여주며, 활액 내 TIMP-2의 감소에 따른 MMPs의 활성이 퇴행성관절염을 더욱 악화시키는 것을 제안해준다. 결국 MMPs의 활성은, 특히 MMP-9, 개의 퇴행성관절염의 조기 진단과 치료를 위한 표지인자로서 사용할 수 있을 것으로 사료된다.

주요어 : MMP, TIMP, 퇴행성관절염, 연골, 활액.