J Vet Clin 20(4) : 443-448(2003)

The Changes of Stifle Joint Fluid with Cranial Cruciate Ligament Rupture in Dogs

Nam-soo Kim[1]

College of Veterianry Medicine, Chonbuk National University
Bio-Safety Research Institute, Chonbuk National University

Abstract : To determine whether localization of tartrate-resistant acid phosphatase (TRAP) and cathepsin K was associated with rupture of the cranial cruciate ligament (CCL) in dogs. Tissue specimens were obtained from 30 dogs with CCL rupture during surgical treatment, 8 aged normal dogs, and 9 young normal dogs that were necropsied for reasons unrelated to this study and unrelated to musculoskeletal disease. The cranial cruciate ligament was examined histologically. $TRAP^+$ cells and cathepsin K^+ cells were identified by histochemical staining and immunohistochemical staining respectively. TRAP and cathepsin K^+ were co-localized within the same cells principally located within the epiligamentous region and to a lesser extent in the core region of ruptured CCL. Localization of $TRAP^+$ cells ($P < 0.05$) and cathepsin K^+ cells ($P = 0.05$) within CCL tissue was significantly increased in dogs with CCL rupture, compared with aged-normal dogs, and young normal dogs ($P < 0.05$ - TRAP, $P < 0.001$ - cathepsin K). Localization of $TRAP^+$ cells and cathepsin K^+ cells within the CCL tissue of aged-normal dogs was also increased compared with young normal dogs ($P < 0.05$). Small numbers of $TRAP^+$ cells and cathepsin K^+ cells were seen in the intact ligaments of aged-normal dogs, which were associated with ligament fasicles in which there was chondroid transformation of ligament fibroblasts and disruption of the organized hierarchical structure of the extracellular matrix. $TRAP^+$ cells and cathepsin K^+ cells were not seen in CCL tissue from young-normal dogs. Localization of the proteinases $TRAP^+$ and cathepsin K^+ in CCL tissue was significantly associated with CCL rupture. Small numbers of proteinase positive cells were also localized in the CCL of agednormal dogs without CCL rupture, but were not detected in CCL from young-normal dogs. Taken together, these findings suggest that the cell signaling pathways that regulate expression of these proteinases in CCL tissue may form part of the mechanism that leads to upregulation of collagenolytic ligament remodeling and progressive structural failure of the CCL over time.

Key words **:** tartrate-resistant acid phosphatase (TRAP), cathepsin K, cranial cruciate ligament (CCL).

Introduction

Rupture of the cranial cruciate ligament (CCL) is one of the most important orthopedic diseases of dogs. Osteoarthritis is usually present in the stifle joint at the time of surgical treatment and tends to get progressively worse after surgery[1]. Progressive deterioration in limb function occurs over time, even with surgical treatment[2]. Complete rupture of the CCL occurs because of progressive structural failure over a period of time in the majority of affected dogs; many of which have bilateral affection[2,3]. Tissue changes that have been identified during progressive CCL rupture in dogs include loss of ligament fibroblasts, transformation of fusiform ligament fibroblasts to an ovoid or spheroid phenotype, and disruption of the normal hierarchical architecture of type I collagen within the extracellular matrix (ECM), including loss of crimp and disruption of ligament fasicles[4,5]. Although various risk factors for CCL rupture have been identified, including aging, body weight, and dog phenotype[6-8], the disease mechanism for CCL rupture in dogs is poorly understood.

After experimental transection of the CCL, approximately 34% of the ligament mass is lost from the CCL by 10 days after surgery[9]. However, the proteinases that are primarily responsible for collagenolysis within CCL tissue after experimental transection have not been determined. Furthermore, the proteinases that initiate collagenolysis during progressive CCL failure in dogs with naturally occurring cruciate disease have not been determined. The loss of collagen mass during remodeling of the CCL after rupture does not appear to be mediated by matrix metalloproteinases[10], and recently, two novel ligament proteinases, tartrate-resistant acid phosphatase (TRAP) and cathepsin K, have been identified in CCL tissue in the dog[11]. These potent proteinases are capable of degrading type I collagen[12,13]. the major structural component of ligament extracellular matrix[14-16]. Their localization within CCL tissue is significantly associated with rupture of the CCL, and with the adaptive remodeling of the CCL that is known to occur with aging[4,5,11]. Localization of TRAP and cathepsin K was not detected in the CCL of young normal dogs[11]. The role of these proteinases, if any, in the disease mechanism for CCL rupture is yet to be determined.

Early detection of cruciate disease in the dog and provision of treatment that prevents development of progressive CCL failure and stifle osteoarthritis is an important goal. Dogs with early cruciate disease tend to have a stable stifle on physical examination, as most of the CCL must be ruptured in order for joint instability to be detected clinically[17,18]. Identification

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

of proteinase activity and the associated ligament remodeling within the CCL may facilitate detection of dogs with preclinical or early cruciate disease and the selection of patients for preventative treatment. The purpose of the present study was to determine whether an association exists between the concentration of TRAP within stifle synovial fluid as a marker for CCL proteinase activity, and localization of TRAP within CCL tissue.

Materials and Methods

Selection of dogs

Portions of CCL and stifle synovial fluid were collected from 30 dogs with CCL disease, which was confirmed at the time of surgical treatment during lateral or medial parapatellar arthrotomy, resection of damaged meniscus, and extracapsular stabilization with nylon suture. Complete CCL rupture was diagnosed if joint instability was detected on physical examination, indicating extensive biomechanical degradation of the CCL. Partial CCL rupture was diagnosed if the affected stifle joint was stable on physical examination. In addition, CCL and stifle synovial fluid specimens were collected from 8 aged dogs and 9 young dogs without CCL disease that were humanely euthanatized by IV administration of barbiturates for reasons unrelated to our study. The group of aged dogs was selected to control for age-related remodeling of the ligament[5,11], and the group of young dogs was selected as a negative control[11]. Age, weight, sex, and duration of lameness for each dog were determined.

Specimen collection and preparation

Remnants of ruptured CCL were excised from the femoral and tibial attachment sites in affected dogs. In dogs with normal CCL ligaments, the entire ligament was collected. Immediately after collection, ligament specimens were placed in tissue cassettes and fixed in Zamboni fixative[19] for 1 to 2 days at 4°C. Longitudinal frozen sections, 10 m thick, were cut and mounted on glass slides for histological examination. Multiple slides were created from each specimen for histochemical and immunohistochemical staining, in addition to staining with H&E. Stifle synovial fluid specimens were also collected at the same time and stored at -80°C for quantification of TRAP activity.

Histochemistry

Histochemical staining specific for TRAP was performed on all CCL specimens, as previously described[11]. All reagents for histochemical staining were obtained from a commercial supplier.a,b Briefly, a solution of naphthol AS-BI phosphate was prepared by dissolving 25 mg of naphthol AS-BI phosphate in 2.5 ml of n-dimethylformamide to which was added 45 ml of 0.05 M Tris-maleate buffer (pH, 5). A solution of hexazotized pararosanaline was prepared by dissolving 0.25 g of pararosaniline hydroxychloride in 5 ml of distilled water, to which was added 1.25 ml of hydroxychloric acid. This solution was mixed with an equal volume of 4% sodium nitrite immediately before use. The final reaction mixture for histochemical staining was prepared by adding 4 ml of hexazotized pararosanaline solution to the naphthol AS-BI phosphate solution, together with 50 mM sodium-potassium tartrate. The final reaction mixture was filtered before use. Sections were incubated in the reaction mixture at 37°C for 1 to 2 hrs, rinsed in distilled water, counterstained in Mayer hematoxylin, and mounted.

All of the ligament specimens were examined via light microscopy for cells that contained TRAP. For each batch of slides, positive and negative controls were also prepared as described[11]. The negative and positive control slides were reviewed before each batch of slides was analyzed.

Synovial Biochemistry.

The concentration of TRAP in stifle synovial fluid was determined using a biochemical assay in 96-well plates[20], with p-nitrophenylphosphate (pNPP) as a substrate. All reagents for this assay were purchased from a commercial supplier.[a,b] All synovial fluid samples were diluted 1:10 in 0.9% NaCl before analysis. Because cells that contained TRAP were not identified in CCL from young normal dogs, the stifle synovial fluid from this group was used as a negative control, and as a diluent for the acid phosphatase standard to correct for background. Acid phosphatase was used as a standard for calculation of the concentration of TRAP within each specimen. Samples were added to the reaction mixture, so that the final incubation medium contained 2.5 mM pNPP (ditris salt), 0.1 M sodium acetate buffer, pH 5.8, 0.2 M KCl, 0.1% Triton X-100, 10 mM sodium tartrate, and the reducing agents ascorbic acid (1 mM) and $FeCl_3$ (100 M). The concentration of TRAP was determined using a 200 µl volume of the final incubation medium per well in the 96-well plate. After incubation of the 96-well plate for 1 hr at 37°C, the p-nitrophenol liberated was converted to p-nitrophenolate by addition of 50 µl of 0.9 M NaOH, and the absorbance was measured at 405 nm using a spectrophotometer.e One unit (U) of TRAP activity corresponds to 1 mol of p-nitrophenol liberated per minute at 37°C. If the concentration of TRAP within a sample exceeded the range of concentrations with the standard curve, the specimen was diluted further and remeasured.

Statistical analyses

The number of cells that contained TRAP, the degree of epiligamentous proliferation, and the degree of chondroid metaplasia within the core region of the CCL were scored for each section by use of a 4-category numerical rating scale (negative = 0; slightly positive = 1; moderately positive = 2; strongly positive = 3). The Median ANOVA was used to determine whether the number of cells that contained TRAP and cathepsin K in CCL tissue was significantly different in dogs with CCL rupture, compared with aged dogs and young dogs with normal stifle joints. Spearman rank correlations were used to examine associations between epiligamentous proliferation,

chondroid metaplasia, and localization of TRAP in dogs with CCL rupture. ANOVA was used to determine the effects of clinical status (aged normal, and CCL rupture) and the number of cells that contained TRAP within CCL on the concentration of TRAP within stifle synovial fluid. Differences were considered significant at $P < 0.05$.

Results

Twenty eight of 30 dogs diagnosed with rupture of the CCL had palpably unstable stifles on physical examination and were diagnosed as having complete tears of the CCL. Partial CCL rupture was confirmed at surgery in the dogs with a stable stifle. Body weight was 35.5 ± 10.8 kg(mean ± SD), and age was 5.2± 2.1 years. Median duration of lameness was 2 months, and lameness duration ranged from 3 days to 24 months. One dog was female, 15 dogs were ovariohysterectomized females, 2 dogs were male, and 12 dogs were castrated males. For the young normal dogs, body weight was 10.0± 0.7 kg, age was 1.7± 0.9 years, and all the dogs were female. For the aged normal dogs, body weight was 28.8 ± 14.8 kg, age was 10 ± 3.7 years. Five dogs were ovariohysterectomized females, and 3 dogs were castrated males(Table 1).

Histologic examination of longitudinal sections of CCL from young normal dogs revealed a dense regularly orientated connective tissue, with parallel bundles of crimped collagen fibers and parallel rows of fusiform ligament fibroblasts. Adjacent to the bone ligament junction, a thin layer of epiligamentous tissue was seen (Fig 1). Mild chondroid metaplasia was seen in the normal CCL from one dog. $TRAP^+$ cells or cathepsin K cells were not seen in normal CCL. Furthermore, TRAP or cathepsin K cells were not seen in the negative controls. In the positive control, $TRAP^+$ and cathepsin K cells were seen on the caudomedial resorption modeling surface of the young rat ulna.

The ligament specimens from the aged normal dogs and dogs with CCL rupture exhibited variable numbers of $TRAP^+$ cells, cathepsin K cells, and chondroid metaplasia within the core region of the CCL. A variable amount of epiligamentous proliferation was also seen in the dogs with CCL rupture. Chondroid metaplasia was significantly increased in aged normal dogs and dogs with CCL rupture, compared with young normal dogs ($P < 0.005$). A significant relationship between duration of lameness and the tissue changes within ruptured CCL was found only for cathepsin K localization, which was increased in dogs with a shorter historical lameness ($P < 0.05$).

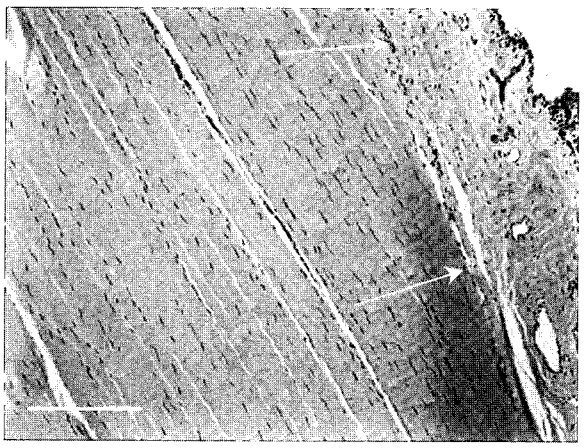

Fig 1. Longitudinal section of normal CCL from a 1 year old female Beagle dog. Hematoxylin and eosin stain, bar = 200 m. Close to the bone-ligament junction, a thin zone of epiligamentous tissue (white arrows) is present surrounding the core region of the ligament, which contains parallel rows of fusiform ligament fibroblasts adjacent to organized crimped ligament fasicles.

Localization of TRAP cells ($P < 0.05$) and cathepsin K cells ($P = 0.05$) within CCL tissue was significantly increased in dogs with CCL rupture, compared with aged normal dogs, and young normal dogs ($P < 0.05$ - TRAP, $P < 0.001$ - cathepsin K). Localization of TRAP cells and cathepsin K cells within the CCL tissue of aged normal dogs was also increased compared with young normal dogs. The TRAP, cathepsin K cells typically had a large rounded phenotype quite different from the fusiform phenotype of the ligament fibroblasts (Fig 2). In dogs with rupture of the CCL, the epiligamentous tissue was much larger in volume and exhibited a high cell number density and blood vessel density. TRAP cells and cathepsin K cells were principally located in the epiligamentous region of the CCL, surrounding the ligament fasicles within the core region. In the aged normal dogs, localization of TRAP and cathepsin K was associated with regions of chondroid metaplasia. The presence of $TRAP^+$ cells was positively correlated with the degree of epiligamentous proliferation ($P < 0.001$) and the presence of cathepsin K cells ($P < 0.01$) (Fig 3). Co-localization of cathepsin K was identified in 16 of 25 dogs with CCL rupture and TRAP cells within the CCL (Fig 3). Furthermore, epiligamentous proliferation was negatively correlated with chondroid metaplasia within the core region of the CCL ($P < 0.005$).

Table 1. Localization of TRAP and cathepsin K in canine cranial cruciate ligament.

Disease Status	B.W(kg)	Age(years)	TRAP+CCL	Cathepsin K+CCL
CCL Rupture (n = 30 dogs)	34.1±12.1	5.1±2.0	28/30[a]	24/30[a]
Young-Normal (n = 9dogs)	10.0± 0.7	1.7±0.9	0/9[b]	0/9[b]
Aged-normal (n = 8 dogs)	30.5±18	9.5±4.3	0/8[b]	0/8[b]

** Mean standard deviation. Rows with differing superscript letters are significantly different ($p < 0.01$).

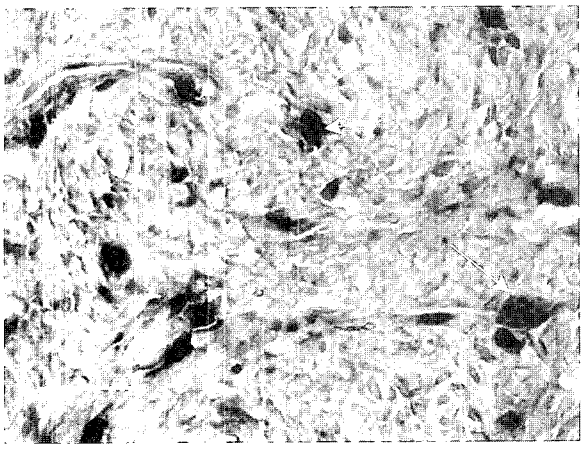

Fig 2. Longitudinal section of the epiligamentous region of ruptured CCL from an 8 years old ovariohysterectomized female Labrador, stained histochemically for TRAP. Mayer's hematoxylin counterstain, bar = 50 m.. The red $TRAP^{+}$ cells (white arrows) typically had a large rounded phenotype, quite different from the predominantly fusiform ligament fibroblasts.

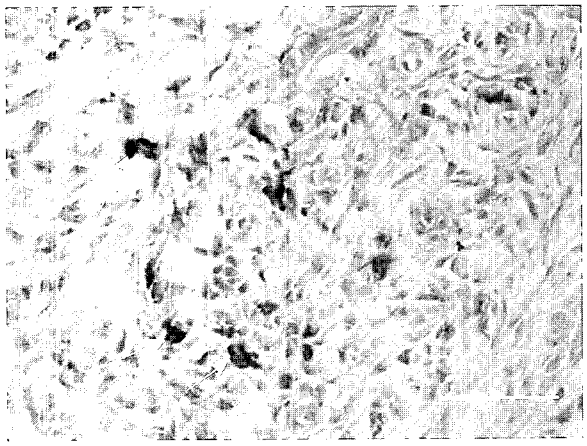

Fig 3. Longitudinal sections of the epiligamentous region of ruptured CCL from an 8 years old ovariohysterectomized female Labrador. The section has been stained immunohistochemically for cathepsin K and counterstained with nuclear fast red, bar = 50 m..

Discussion

Rupture of the CCL is one of the most common and important orthopedic diseases of pet dogs, and a rich body of literature is available describing the clinical features and surgical treatment of this condition. However, although it is generally accepted that the majority of the CCL ruptures are not associated with a primary traumatic injury, the underlying disease mechanism for CCL rupture is poorly understood. It has been previously hypothesized that expression of proteinase within the CCL during injury may have an important role in CCL resorption and the success of reconstructive surgery[13]. In the present study, we have shown that localization of the proteinases TRAP and cathepsin K are significantly associated with rupture of the CCL. Small numbers of proteinase positive cells were also seen in the CCL of aged normal dogs, principally in ligament fasicles with chondroid degeneration and in the epiligament.

TRAP belongs to a group of iron-binding proteins that includes uteroferrin and purple acid phosphatase, and is localized intracellularly in the lysosomal compartment of osteoclasts, macrophages, and dendritic cells[16]. TRAP also may be secreted by some cell types, in particular by osteoclasts[16]. TRAP is widely expressed in a range of tissues, including bone, spleen, liver, thymus, and colon, where it is associated principally with cells originating from the bone marrow[17]. The process of osteoclastic bone resorption including collagenolysis is mediated by TRAP[18]. However, the functional role of TRAP in tissues other than bone is unknown. Cathepsin K is a cysteine proteinase that is expressed primarily by osteoclasts[19]. The enzyme has a key role in osteoclastic bone resorption and is a potent proteinase of type I collagen[20]. Recently, co-expression of TRAP and cathepsin K has been identified in CD68+ macrophages involved in bone resorption[21].

TRAP and cathepsin K are two proteins necessary for the formation of activated osteoclasts and normal bone resorption[26,27] In knockout mice, deletion of these genes leads to the development of osteopetrosis[26,27]. Cathepsin K is a potent proteinase of type I collagen and is the principal proteinase acting on bone collagen during osteoclastic bone resorption[26], and during resorption of bone by CD68+ macrophages[21]. Cathepsin K is secreted as a pro-enzyme and when activated cleaves type I collagen at the N-terminal end of the triple helix[28,29]. TRAP also is secreted as a pro-enzyme and may be activated by cysteine proteinases[30], such as cathepsin K. Although the precise role of TRAP in osteoclastic bone resorption is not well understood, it is thought that TRAP may facilitate resorption of bone by generation of reactive oxygen species that non-specifically degrade bone collagen[31]. TRAP may be inhibited by binding with 2-macroglobulin[32]. The precise role of TRAP and cathepsin K expression in remodeling of ligament collagen, and the cell signaling pathways regulating their expression within ligament tissue are unknown. Up-regulation of these enzymes in ruptured CCL and to a lesser extent in the CCL of aged-normal dogs suggests that their presence within CCL tissue may form part of the mechanism that leads to progressive structural failure of the CCL[33]. Detection of these proteinases in the CCL of aged normal dogs suggests that it is less likely that localization of these novel proteinases within the CCL may be secondary to the primary rupture mechanism, and that they are simply involved in the epiligamentous remodeling response after CCL rupture. Important objectives in future research will be to determine that these enzymes cause collagenolysis of the ECM; whether upregulation of these proteinases causes structural weakening of the CCL; and what are the signaling pathways that regulation expression of these enzymes in CCL tissue.

The dogs with CCL disease in this study had signalments that were typical for this condition[8,9] and were generally larger, middle aged dogs. Middle aged large breed dogs often have mild stifle osteoarthritis, and are known to have characteristic changes to the phenotype of CCL fibroblasts as well as the ligament ECM[5], as was identified in the aged normal dogs of the present study. The presence of small numbers of proteinase positive cells in the CCL of aged normal dogs with chondroid degenerative change suggests that these changes may represent pre-clinical CCL disease. In this initial study, we also determined localization of TRAP and cathepsin K in the CCL of young normal dogs principally as a control for validation of the specificity of our histochemical and immunohistochemical staining. Although all of our normal dogs were female, gender is not a significant risk factor for CCL rupture[9]. The complete absence of TRAP and cathepsin K cells within the CCL from this population of young-normal dogs also supports our working hypothesis that localization of these enzymes within ligament tissue is associated with upregulation of collagenolytic remodeling within diseased CCL. Lameness in dogs with cruciate disease is likely caused by both joint instability and osteoarthritis. Furthermore, the ligament mechanical properties are probably degraded over time[5]. We believe that these factors are the likely explanation for the lack of correlation between the presence of TRAP cells within the ruptured CCL and the duration of lameness in the dogs of this study. The increased localization of cathepsin K within the CCL of dogs with a short duration of lameness suggests cathepsin K may be associated with late ligament remodeling and final structural failure.

We identified co-localization of cathepsin K in 64% of ruptured CCL containing $TRAP^+$ cells. This value probably underestimates co-localization because of false negative double-staining in some ligaments, which contained only a small quantity of cathepsin K^+ cells. Furthermore, some sections from dogs with CCL rupture contained tissue predominantly from the core region, which had marked phenotypic transformation of ligament fibroblasts[4,5], and little epiligamentous tissue. The precise origin of the TRAP+, cathepsin K cells within diseased CCL is unclear. Although it is recognized that ligament tissue adapts and remodels to its loading enviroment[11], this process is poorly understood. In particular, it is not known what type of cell is primarily responsible for collagenolysis during non-inflammatory ligament remodeling. Furthermore, the role of the epiligamentous remodeling response, where the TRAP and cathepsin K cells predominate, in the CCL rupture mechanism also is unclear. Although it has been previously suggested that ligament fibroblasts may be primarily responsible for collagenolysis during remodeling[14], the rounded, as opposed to fusiform, phenotype of these TRAP, cathepsin K cells, and the recent discovery that CD68, TRAP, cathepsin K macrophages[21] are capable of osteolysis, suggests that the TRAP, cathepsin K cells we have identified in diseased CCL may be bone marrow-derived macrophage-like cells that migrate into CCL tissue in a process analogous to the activation of osteoclastic bone resorption.

Conclusion

We have identified cells with a rounded phenotype within CCL tissue from dogs with CCL rupture and aged normal dogs without CCL rupture that co-express the proteinases TRAP and cathepsin K. The significant association between the presence of these proteinases within CCL tissue and CCL rupture suggests that the enzymes may be involved in the cellular events associated with CCL rupture, repair, or both. It seems possible that the cell signaling pathways that regulate expression of these enzymes may form part of the mechanism that leads to upregulation of collagenolytic ligament remodeling and progressive structural failure of the canine CCL over time. But We did not detect these rounded proteinase positive cells in not CCL tissue from young-normal dogs.

Acknowledgement

This study was supported by a grant from the Korea Science Engineering Foundation (KOSEF, 2001 Post-Doctor support) grant fund.

References

1. Vasseur PB, Berry CR. Progression of stifle osteoarthrosis following reconstruction of the cranial cruciate ligament in 21 dogs. J Am Anim Hosp Assoc 1992; 28: 129-136.
2. Innes JF, Bacon D, Lynch C, Pollard A. Long-term outcome of surgery for dogs with cranial cruciate ligament deficiency. Vet Rec 2000; 147: 325-328.
3. Bennett D, Tennant B, Lewis DG, et al. A reappraisal of anterior cruciate ligament disease in the dog. J Small Anim Pract 1988; 29: 275-297.
4. Narama I, Masuoka-Nishiyama M, Matsuura T, et al. Morphogenesis of degenerative changes predisposing dogs to rupture of the cranial cruciate ligament. J Vet Med Sci 1996; 58: 1091-1097.
5. Vasseur PB, Pool RR, Arnozky SP, Lau RE. Correlative biomechanical and histologic study of the cranial cruciate ligament in dogs. Am J Vet Res 1985; 46: 1842-1854.
6. Whitehair JG, Vasseur PB, Willits NH. Epidemiology of cranial cruciate ligament rupture in dogs. J Am Vet Med Assoc 1993; 203: 1016-1019.
7. Duval JM, Budsberg SC, Flo GL, Sammarco JL. Breed, sex, and body weight as risk factors for rupture of the cranial cruciate ligament in young dogs. J Am Vet Med Assoc 1999; 215: 811-814.
8. Morris E, Lipowitz AJ. Comparison of tibial plateau angles in dogs with and without cranial cruciate ligament injuries. J Am Vet Med Assoc 2001; 218: 363-366.
9. Amiel D, Ishizue KK, Harwood FL, et al. Injury of the anterior cruciate ligament: The role of collagenase in ligament degeneration. J Orthop Res 1989; 7: 486-493.
10. Spindler KP, Clark SW, Nanney LB, Davidson JM. Expression of collagen and matrix metalloproteinases in ruptured human anterior cruciate ligament: An in situ

hybridization study. J Orthop Res 1996; 14: 857-861.
11. Muir P, Hayashi K, Manley PA, Colopy SA, Hao Z. Evaluation of tartrate-resistant acid phosphatase and cathepsin K in ruptured cranial cruciate ligaments in dogs. Am J Vet Res 2002; 62: 228-235.
12. Petersen W, Tillmann B. Structure and vascularization of the cruciate ligaments of the human knee. Anat Embryol 1999; 200: 325-334.
13. Amiel D, Frank C, Harwood F, et al. Tendons and ligaments: A morphological and biochemical comparison. J Orthop Res 1984; 1: 257-265.
14. Bossard MJ, Tomaszek TA, Thompson SK, et al. Proteolytic activity of human osteoclast cathepsin K. J Biol Chem 1996; 271: 12517-12524.
15. Kafienah W, Bromme D, Buttle DJ, et al. Human cathepsin K cleaves native type I and II collagens at the N-terminal end of the triple helix. Biochem J 1998; 331: 727-732.
16. Halleen JM, Raisanen S, Salo JJ, et al. Intracellular fragmentation of bone resorption products by reactive oxygen species generated by osteoclastic tartrate-resistant acid phosphatase. J Biol Chem 1999; 274: 22907-22910.
17. Heffron LE, Campbell JR. Morphology, histology and functional anatomy of the canine cranial cruciate ligament. Vet Rec 1978; 102: 280-283.
18. Scavelli TD, Schrader SC, Matthiesen DT, Skorup DE. Partial rupture of the cranial cruciate ligament of the stifle in dogs: 25 cases (1982-1988). J Am Vet Med Assoc 1990; 196: 1135-1138.
19. Stefanini M, De Martino C, Zamboni L. Fixation of ejaculated spermatozoa for electron microscopy. Nature 1967; 216: 173-174.
20. Lang P, Schultzberg M, Andersson G. Expression and distribution of tartrate-resistant acid purple acid phosphatase in the rat nervous system. J Histochem Cytochem 2001; 49: 379-396.
21. Konttinen YT, Takagi M, Mandelin J, et al. Acid attack and cathepsin K in bone resorption around total hip replacement prosthesis. J Bone Min Res 2001; 16: 1780-1786.
22. Foged NT, Delaisse JM, Hou P, et al. Quantification of collagenolytic activity of isolated osteoclasts by enzyme-linked
23. Van de Wijngaert FP, Burger EH. Demonstration of tartrate-resistant acid phosphatase in un-decalcified, glycomethacrylate-embedded mouse bone: A possible marker for (Pre) Osteoclast identification. J Histochem Cytochem 1986; 34: 1317-1323.
24. Gomori G. Microscopic histochemistry: Principles and Practice. Chicago, IL, University of Chicago Press, 1952: 137-221.
25. Hillam RA, Skerry TM. Inhibition of bone resorption and stimulation of formation by mechanical loading of the modeling rat ulna in vivo. J Bone Miner Res 1995; 10: 683-689.
26. Saftig P, Hunziker E, Wehmeyer O, et al. Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice. Proc Natl Acad Sci 1998; 95: 13453-13458.
27. Hayman AR, Jones SJ, Boyde A, et al. Mice lacking tartrate-resistant acid phosphatase (Acp 5) have disrupted endochondral ossifica.
28. Lamp EC, Drexler HG. Biology of tartrate-resistant acid phosphatase. Leuk Lymphoma 2000; 39: 477-484.
29. Hayman AR, Bune AJ, Bradley JR, et al. Osteoclastic tartrate-resistant acid phosphatase (Acp 5): Its localization to dendritic cells and diverse murine tissues. J Histochem Cytochem 2000; 48: 219-227.
30. Ljusberg J, Ek-Rylander B, Andersson G. Tartrate-resistant purple acid phosphatase is synthesized as a latent proenzyme and activated by cysteine proteinases. Biochem J 1999; 343: 63-69.
31. Oddie GW, Schenk G, Angel NZ, et al. Structure, function, and regulation of tartrate-resistant acid phosphatase. Bone 2000; 27: 575-584.
32. Shaffer Brehme C, Roman, S, Shaffer J, Wolfert R. Tartrate-resistant acid phosphatase forms complexes with 2-macroglobulin. J Bone Miner Res 1999; 14: 311-318.
33. Murray MM, Martin SD, Martin TL, Spector M. Histological changes in the human anterior cruciate ligament after rupture. J Bone Joint Surg 2000; 82A: 1387-1397.

개에 있어서 전방십자인대 단열시 슬관절액의 변화

김 남 수[1]

전북대학교 수의과대학, 전북대학교 생체안전성연구소

요 약 : 본 연구는 전십자인대가 단열된 개의 슬관절액에서 tartrate-resistant acid phosphatase (TRAP)와 cathepsin K의 변화를 알아봄으로서 십자인대에 관련된 질병 또는 십자인대 치료 실패에 따른 퇴행성관절염의의 조기 진단 및 치료에 관한 정보를 얻기 위하여 실시하였다. 실험동물로는 전방십자인대가 단열된 30두의 개와 정상 성견 8두 그리고 어린 정상 개 9두를 사용하였다. 슬관절액 내의 TRAP과 Cathepsin K의 변화를 확인하기 위하여 조직화학 염색과 면역조직화학염색을 실시하였다. 조직학적인 검사 결과 정상견에서는 TRAP과 Cathepsin K 세포들은 찾아보기 힘들었으나 전방십자인대가 단열된 개에 있어서는 TRAP과 Cathepsin K 그리고 chondroid metaplasia가 중심부분에서 증가되고 있음을 관찰하였다. 이러한 결과로 보아 TRAP과 Cathepsin K 세포들은 전방십자인대가 단열 또는 단열 후 회복되는 과정에서 상호 보조적으로 관여하며 세포외 방출과 관계가 있는 것으로 생각된다.

주요어 : 전방십자인대 단열, tartrate-resistant acid phosphatase (TRAP), cathepsin K, 슬관절액.