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The Concentration of Tartrate Resistant Acid Phosphatase in Synovial Fluid of Canine Stifle Joint

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Abstract: The concentration of tartrate resistant acid phosphatase (TRAP) in synovial fluid of normal stifle joint was investigated in order to establish a baseline data to distinguish between healthy joint and joint with injury of cranial cruciate ligament (CCL). Twenty three mixed-breed healthy dogs free from joint diseases (fourteen adult and nine young) were used in this study. The dogs were sedated and synovial fluid was collected from the femoropatellar compartment of stifle joints by direct arthrocentesis. The concentration of TRAP in synovial fluid was determined using the method of Lang. The concentration of TRAP were 0.083±0.039 IU/ml in adult dogs, 0.064±0.023 IU/ml in young dogs, 0.075 ±0.028 IU/ml in large dogs (>22 kg), 0.076±0.046 IU/ml in small dogs (<22 kg), 0.085±0.036 IU/ml in neutered dogs and 0.056±0.022 IU/ml in intact dogs. The concentration of TRAP in the neutered dogs was significantly ($p<0.05$) higher than the intact dogs. This data can be used baseline data for a comparison with joint with injury of cranial cruciate ligament.

Key words : Tartrate resistant acid phosphatase, synovial fluid, Cranial cruciate ligament

Introduction

Injury of cranial cruciate ligament (CCL) is one of the most important causes of osteoarthritis (OA) of the stifle joint in dogs[3,4]. In most injury of CCLs, progressive deterioration in joint function over time is common and usually leads to the development of persistent lameness after surgical or medical treatment. It is well recognised that the severity of CCL disease is identified by physical examination or radiography. But mild CCL disease and partial CCL tears may exist in small animal in the absence of any clinical sign, not typically detected radiographically[9,16]. Mild CCL disease and partial CCL tears that are treated medically with the adminstration of analgesics and restriction of exercise often progress to complete tears over time. The early diagnosis of CCL disease is very important for the prevention of complete rupture of CCL.

Muir et al[15] reported that tartrate-resistant acid phosphatase (TRAP) is biomarker of altered CCL metabolism. These protease have potent extracelluar collagenolysis activity[1,5,12]. Injury of the CCL is associated with localization of the proteinase TRAP within CCL tissue on immunohistochemical staining, especially in the epiligamentous tissue surrounding remodeling ligament fascicles[15]. The expression of proteinase TRAP within the CCL during injury has an important role in CCL resorption and remodeling and TRAP released from injury of CCL into synovial fluid[11,15]. The baseline data of the concentration of TRAP in normal stifle joint is thought to give important information when compared to that incase of injury of CCL. Furthermore, it facilitates early diagnosis of CCL disease such as partial tears. But, there is no avaliable baseline data on the concentration of TRAP to compare with CCL disease.

The purpose of this study was to investigate the concentration of TRAP in synovial fluid of normal stifle joint and distinguish it from age, body weight and sex in order to establish a baseline data, so that it can be compared with that obtained in case of injury of cranial cruciate ligament.

Materials and Methods

Experimental animals

Twenty three mixed breed dogs of both sexes were used in this study. All the dogs were healthy and free from systemic and joint diseases. Each dog was examined physically and radiographically to observe the stability of the stifle joint and make sure that the dogs have no CCL disease and finally it was confirmed through an exploratory joint surgery after collection of the synovial fluid.

Collection of synovial fluid

The dogs were sedated with 1.1 mg/kg intramuscularly (Rompun®, Bayer Korea Ltd, Korea) and placed on lateral recumbency with the stifle joint flexed. Digital pressure was applied to medial side of straight patellar ligament and 19 to 21 gauge needle inserted with 5 ml plastic syringe was directed into space, through the fat pad, towards the intercondylar space lateral to the straight patellar ligament. The synovial fluid was collected as much volume (0.1-0.4 ml) as possible and special care was taken to avoid contamination

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with blood. After collection, the synovial fluid was centrifuged at 12,000 × g for 10 minutes at 4°C to remove cells, and the supernatant was stored at -80°C until assayed.

Assessment of TRAP activity

The concentration of TRAP in stifle synovial fluid was determined using method of Lang in 96-well plates, with p-nitrophenylphosphatate (pNPP) (Sigma Chemical Co, St Louis, USA) as substrate[5]. All synovial fluid samples were diluted 1:10 in 0.9% NaCl before analysis. 0.9% NaCl was used as 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.

A 100 μl of samples were added to the TRAP buffer which were made 2.5 mM pNPP (ditris salt), 0.1 M sodium acetate buffer, 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 incubation medium per 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 ELISA (Cess UV 90c, Bioteck co, USA). One unit 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

Concentration of TRAP were reported as mean ± SD. The data obtained was analysed using unpaired Student's t-test and the differences were considered significant when p values under <0.05.

Results

Age

The concentration of TRAP in adult dogs (>2 year) was 0.083 ± 0.039 IU/ml(mean ± SD) which was higher in comparison to that of the young dogs (<2 year), 0.064 ± 0.023 IU/ml(mean ± SD). But this difference was not statistically significant (p>0.05). The concentration of TRAP in adult and young dogs is presented in Fig 1.

Body weight

The concentration of TRAP in the larger dogs (>22kg) was 0.075 ± 0.028 IU/ml(mean ± SD) and that in the smaller dogs (< 22kg) was 0.076 ± 0.046 IU/ml(mean ± SD) which was almost equal (Fig 1).

Sex

The concentration of TRAP in the male dogs was 0.072 ± 0.028 IU/ml(mean ± SD) and that in the females was 0.077 ± 0.038 IU/ml(mean ± SD). The concentration of TRAP in the females was higher when compared to that in the males, but this difference was not statistically significant (p>0.05). The result is shown in Fig 3.

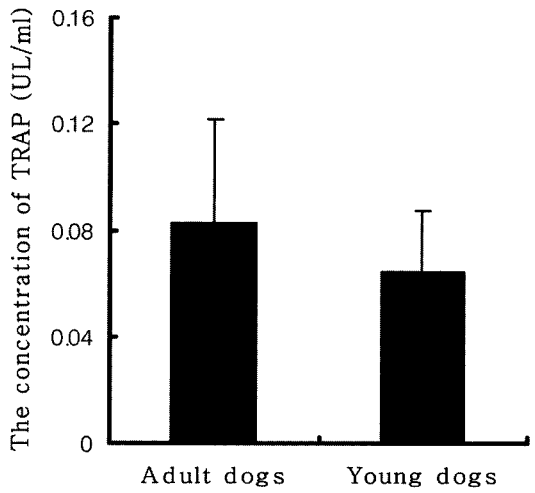

Fig 1. The concentration of TRAP in 14 adult and 9 young dogs.

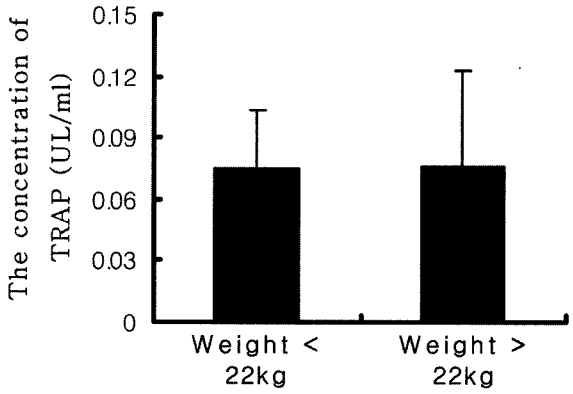

Fig 2. The concentration of TRAP in 15 larger (>22 kg) and 8 smaller (<22 kg) dogs.

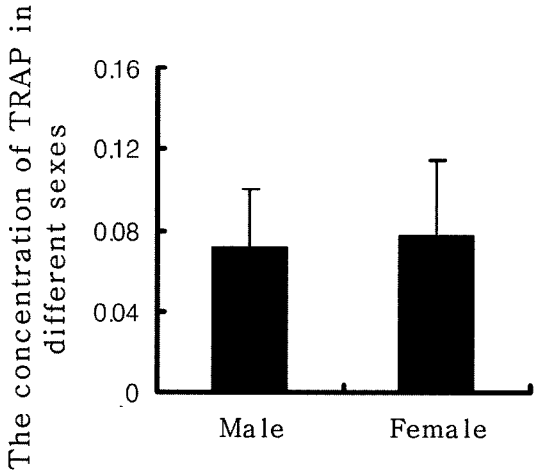

Fig 3. The concentration of TRAP in male and female dogs.

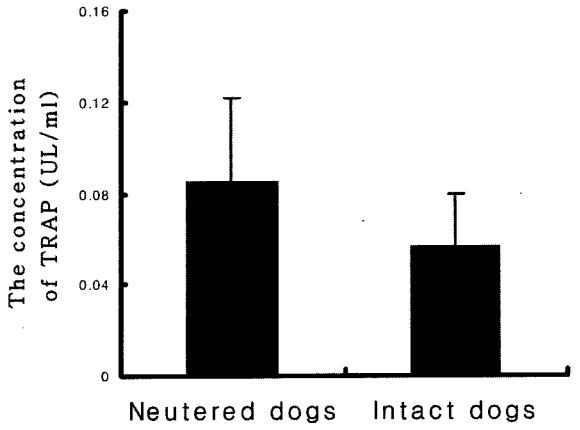

Fig 4. The concentration of TRAP in 15 neutered and 8 intact dogs.

The concentration of TRAP in the neutered dogs was 0.085 ± 0.036 IU/ml(mean ± SD), which was significantly ($p<0.05$) higher when compared to that in the intact dogs 0.056 ± 0.022 IU/ml(mean ± SD). The result is presented in Fig. 4.

Discussion

Tartrate-resistant acid phosphatase localized intracellular in the lysosomal compartment of osteoclasts. More recent studies using sensitive immunocytochemical and RT-PCR techniques have shown that TRAP is expressed in diverse tissues harboring cells of bone marrow origin, including dendritic cells, activated macrophages and cells belonging to osteoclast, macrophage lineage[8,12,14]. However, osteoclasts are still the most major cells that express TRAP[7].

TRAP has been used for several years as a marker enzyme of bone-resorption osteoclasts. The process of osteoclastic bone resorption, inculding collagenolysis, is mediated by TRAP, but, the functional role of TRAP in tissue other than bone is unknown. Recently, co-expression of TRAP and cathepsin K has been identified in CD68+ macrophages involved in bone resorption[12,13,15]. Dogs with CCL rupture and aged dogs without CCL rupture had TRAP positive cells in CCL tissue[15]. The significant association between the presence of this proteinase and ruptured CCL suggests that the expression of proteinase TRAP within the CCL during injury have an important role in CCL resorption and repair, or both[15].

Many of the previous authors reported that with aging, the CCL undergoes a degenerative process that is characterizes by the loss of normal fiber bundle morphology and dystrophic mineralization[4,6]. The CCL tissue of aged dogs also has small number of TRAP positive cells of which play the role expression in remodeling of ligament collagen[15]. However, in our study we did not find any significant difference between the concentration of TRAP in adult and young dogs.

Dogs weighing more than 22 kg were reported to have an increased risk for injury to the CCL. Histologically, ligament from larger dogs between 5 and 7 years old have evidence of degeneration whereas, ligaments from smaller dogs do not have degenerative changes unless the dogs are between 7 and 11 years old. However, in our study we did not find any significant difference between the concentration of TRAP in larger and smaller dogs. We thought that body weight might not be the sole factor for contributing to the CCL injury. There are several breeds of dogs weighing <22 kg which have a higher prevalence of injury of CCL. For example, the Rottweiler and the Labrador Retriever are considered to be conformationally straight in the hid limbs, compared with other breeds, which may contribute to stress on the CCL such as hyperextension of the stifle. Conformational abnormalities may cause excessive loading of CCL and contribute to deterioration and failure of ligament.

The prevalence of injury of CCL is reported to be higher in neutered dogs of both sexes, compared with the sexually intact dogs. A significant difference in the concentration of TRAP in synovial fluid between neutered and intact dogs of both sexes was observed in our study ($p<0.05$). Why this difference occurs is not clear, but it might be due to change in the endocrine system following their neutralization. Abnormal weight gain increase the load on musculoskeletal structures and could accelerate degenerative processes in the CCL. Histochemistry, morphological method have been used to demonstrate that connective tissue metabolism is influenced by the endocrine system. Ovariectomy in beagles caused persistent hypoestrogenemia, a 68% increase in the rate of trabecular bone remodeling, and a net loss of bone mass[2]. The effect of hypoestrogenemia on other connective tissue is not well defined. Estrogen inhibits collagen synthesis in the arota of rats, rat tail tendon, and mouse skin, whereas it accelerates collagen synthesis in the uterus of rat. Ovariectomy in rats was shown to decrease the elastin content and fiber diameter in the hip joint capsule. Diagnosis of injury of CCL may be delayed for many reasons, including difficulties in the clinical diagnosis of partial tears, gradual onset of lameness and concurrent abnormalities such as hip dysplasia. The early diagnosis of some CCL diseases such as partial rupture or tear are difficult because of the absence of obvious clinical signs and lameness. Radiographs are also equivocal. However, in such cases measurement of the concentration of TRAP in synovial fluid might play an important role in their diagnosis and treatment.

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정상 개의 관절액에서 TRAP(Tartrate resistant acid phosphatase)농도 측정

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요 약: 본 연구에서는 십자인대 손상견의 무릎관절액의 TRAP의 농도와 비교 할 수 있는 정상 관절액의 TRAP 농도의 지표를 제시 하기 위하여, 정상 무릎 관절액의 TRAP의 농도를 측정하였다. 십자인대 손상이 없는 23두 건강한 잡종견(14두 노령견과 9두 성견)을 실험견으로 선택하고 성별, 나이, 몸무게로 구별하였다. 무릎 관절액을 채취 한뒤, Lang method로 관절액의 TRAP을 측정하였다. 측정된 TRAP의 농도를 성별, 나이, 몸무게 등으로 구별하여 Mean±SD로 표시 하였으며, 유의성 차이를 T-test로 구별하였다. 노령견(14두, 2년이상) TRAP의 농도는 0.083±0.039 IU/ml (mean±SD), 성견(9두, 2년 이하) 0.064±0.023 IU/ml, 대형견(15두, >22 kg) 0.075±0.028 IU/ml, 소형견(8두, <22 kg) 0.076±0.046 IU/ml, 중성화견(15두) 0.085±0.036 IU/ml, 중성화 되지 않은견(8두) 0.056±0.022 IU/ml (Mean±SD)로 나타났으며, 중성화 견과 중성화 되지 않은 견에서만 유의성 있는 차이 ($P<0.05$)가 보였다. 본 연구의 정상견에서 TRAP 농도는 십자인대의 손상견과 비교할수 있는 지표로 활용할 수 있을 것으로 사료된다.

주요어: Tartrate resistant acid phosphatase, 관절액, 십자인대