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# Efficacy of Intraarticular Administration of Hyaluronic Acid in Osteoarthritis After Surgical Correction of Canine Cranial Cruciate Ligament Rupture

Natee Techaarpornkul<sup>1</sup> Kumpanart Soontornvipart<sup>1\*</sup> Prach Maihasap<sup>1</sup>

## *Abstract*

Canine osteoarthritis is a common disease in clinical practice. Causes of canine osteoarthritis are obscure. At present, nutraceuticals and hyaluronic acid are used for the treatment of canine osteoarthritis. This study assessed the efficacy of intraarticular administration of hyaluronic acid on the treatment of canine osteoarthritis. After undergoing surgical correction of cranial cruciate ligament rupture, 16 dogs were divided into 2 groups, HA and control groups. The dogs in the HA group (n=8) received intraarticular injection of hyaluronic acid. The control group (n=8) did not receive hyaluronic acid treatment after surgery. Lameness score, weight bearing score, radiographic examination and serum OA biomarker (WF6) were used for analysis. Results revealed that the HA group had better lameness score and weight bearing score, but there was no statistic difference in the radiographic finding and the levels of serum WF6 after 8 weeks of treatment.

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**Keywords:** cranial cruciate ligament, hyaluronic acid, nutraceutical, osteoarthritis, WF6

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## Introduction

Osteoarthritis is caused by degeneration of the cartilage surface without inflammatory mechanism. Additionally, this circumstance mostly emerges with osteophyte, capsular fibrosis, subchondral bone sclerosis and synovial tissue, respectively (Todhunter and Johnston, 2003). Although currently the causes of osteoarthritis have not yet been found, the most possible factors are breed, age, food, hormone, weight and abnormal weight bearing (Ghosh, 1999; Felson, 2004).

Cranial cruciate ligament disease is one of the symptoms mostly found in dogs, particularly in aged dogs. This condition can cause osteoarthritis in long-term. At present cranial cruciate ligament disease is treated by surgery in order to make the knee stable and reduce the chance of osteoarthritis (Vasseur, 2003).

There are several equipment and technologies for the diagnosis of osteoarthritis such as Magnetic Resonance Imaging (MRI) including laboratory and biomarker. For the biomarker, this method measures the molecule or compound eliminated into body fluid by immunoenzymatic technique. The advantage of this method is that anomaly can be found since the beginning of formation of molecule (Caterston et al., 1995; Garnero, 2006; Garvican et al., 2010a; Garvican et al., 2010b).

Biomarker WF6 is the indicator of specific component of cartilage that is an indicator of degradation of chondroitin sulfate. In this process, epitope of WF6 is gathered with WF6 monoclonal antibody. This antibody is crucial to chondroitin-6-sulfate and chondroitin 2,6 disulfate using native chondroitin sulfate epitope (Nganvongpanit and Ong-Chai, 2004). Monoclonal WF6 is researched by Bone and Joint Research Laboratories, Faculty of Medicine, Chiangmai University.

The use of medicine and nutrition pharmacy products is a common treatment for osteoarthritis. Basically, nonsteroidal anti-inflammatory drugs (NSAIDs) is one kind of medicine used to treat osteoarthritis. Even though this kind of medicine directly reduces pain and inflammation, it has some side effects on animals. Consequently, using nutraceutical may be the proper choice for curing osteoarthritis. Moreland (2003) claimed that hyaluronic acid (HA), which is the inherent component of synovial fluid, is one of the chosen products to shield animals from osteoarthritis. The components of this product also act as lubricating substance and prevent inflammatory cell into the joint. HA for osteoarthritis treatment is mostly in the form of intraarticular injection. Hence, Viscosupplementation is named instead of nutraceutical.

When osteoarthritis occurs, quantity and molecular weight of hyaluronan are decreased, causing reduction in viscosity of synovial fluid (Nganvongpanit, 2008; Todhunter and Johnston, 2003). There are various researches that used several forms of hyaluronic acid to treat osteoarthritis (Hulmes et al., 2004; Wang et al., 2004; Gomis et al., 2007; Strauss et al., 2009). As a result, hyaluronic acid gave effective treatment for osteoarthritis. Some researches, however, indicated that hyaluronic acid had no effect or negative

effect on the treatment of osteoarthritis (Gonzalez-Fuentes et al., 2010; DeGroot et al., 2012).

However, most research into hyaluronic acid used hyaluronic more than one time except the research of Nganvongpanit et al. (2013), which studied the intraarticular use of hyaluronic acid after stifle joint surgery and found that hyaluronic acid was able to decrease the chance of occurring osteoarthritis when compared with control group in 1 month. This research also utilized biomarker in order to measure the result.

Consequently, this study aimed to demonstrate the efficacy of hyaluronic acid in osteoarthritis after correction of cruciate ligament rupture by the study of biomarker, clinical assessment and radiological study. Results of this study will be useful for selection of medical treatment after joint surgery in human and animal.

## Materials and Methods

**Animals:** Sixteen dogs, weighing 15-30 kilograms and aged 2-10 years, were presented at Small Animal Teaching Hospital Chulalongkorn University with unilateral cranial cruciate rupture disease. All protocols used in this study were approved by the Committee of the Ethical Care of Animal of Chulalongkorn University.

**Groups:** The dogs were divided into 2 groups by double blind randomized technique into treatment group and control group. Eight dogs in the treatment group received hyaluronic acid after surgery. Eight dogs in the control group did not received any solution.

**Surgical procedure:** In anesthetic protocol, all dogs received Acepromazine (Combistress®) at a dose of 0.02-0.03 mg/kg with morphine at a dose of 0.5 mg/kg by intramuscular injection as a premedication. After lethargy, Acetar solution was given to the dogs as intravenous fluid. After that propofol (Lipuro®) at a dose of 4 mg/kg was intravenously injected as induction. The unconscious dogs were then intubated with an endotracheal tube and anesthesia was maintained with isoflurane 1.5-2 percent until surgery finished.

Before surgery all dogs received cefazolin (Zefa M.H.®) at a dose of 25 mg/kg intravenously as prophylaxis antibiotic. The dogs in both groups received surgical treatment by the same surgeon with extracapsular stabilization technique with 100 lb CCL suture.

After closing the joint capsule, the dogs in the treatment group received hyaluronic acid intraarticularly, while the dogs in the control group did not receive any.

Hyaluronic acid in this study was 1% hyaluronic acid sodium salt (Hyalgan®) with a molecular weight of 500-730 kDal specifically for intraarticular injection.

After the surgical procedure, all dogs received cephalixin (Toflex®) at a dose of 25 mg/kg bid PO for 7 days as antibiotic, caprofen (Rimadyl®) at a dose of 4 mg/kg sid PO for 4 days and tramadol

hydrochloride (Anadol®) at a dose of 4 mg/kg bid PO for 5 days as analgesic drugs.

**Clinical assessment:** All dogs underwent physical examination and clinical scoring system (McCarthy et al., 2007) before surgery and on weeks 2, 4 and 8 after surgery. McCarthy's clinical scoring system is composed of lameness score and weight bearing score that range from grade I-V. The lameness scores are as follows: Grade I means dog can walk normally, Grade II means dog can walk with mild lameness, Grade III means dog can walk with moderate lameness, Grade IV means dog can walk with severe lameness and Grade V means dog cannot walk more than 5 steps and struggle to stand. The weight bearing scores are as follows: Grade I means dog stand normally, Grade II means dog stand normally but with mild abnormal bearing when walk, Grade III means partial weight bearing when stand and walk, Grade IV means partial weight bearing when stand and no weight bearing when walk, and Grade V means no weight bearing when stand and walk. The assessment was done by 2 same clinicians for all examples.

**Radiological study:** All samples were taken for radiographic examination in anterior-posterior and medio-lateral posture on affected stifle joint before surgery and on weeks 2, 4 and 8 after surgery. Kellgren-Lawrence scoring system was used for assessment. Grade 0 means not find any abnormality, Grade I means doubtful, Grade II means find minimal osteophyte, Grade III means find multiple sites of osteophyte and capsular fibrosis and may find bone deformity, and Grade IV means find large osteophyte with severe capsular fibrosis and bone deformity (Kellgren and Lawrence, 1957; Takahashi et al., 2004). The assessment was done by 2 same radiologists for all examples.

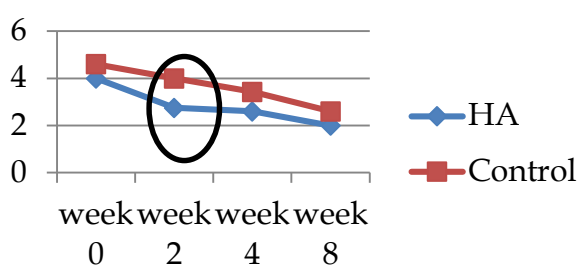
**Serological examination:** One milliliter of blood was collected from the cephalic vein or lateral saphenous vein for complete blood count and blood chemistry before surgery and on weeks 2, 4 and 8 after surgery.

**Serum biomarker WF6:** Four milliliters of blood was collected for WF6 before surgery and on weeks 2, 4 and 8 after surgery to examine serum WF6 by Enzyme-Linked Immunosorbent Assay (ELISA). By using the competitive inhibition ELISA method, serum WF6 was captured by monoclonal antibody WF6, a primary antibody, then the compound was coated with embryonic shark skeletal cartilage aggrecan to be used as antigen. After the antigen was put on a microplate conjugated anti-mouse IgM antibody, as a secondary antibody, was filled to examine serum WF6.

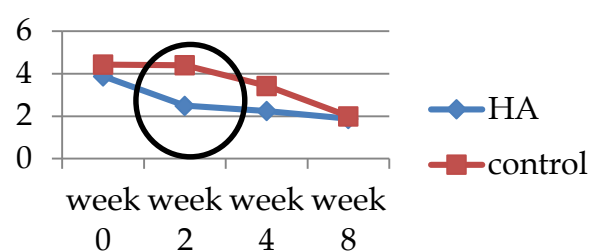
**Statistical analysis:** Signalment, serological examination and health status were analyzed in descriptive analysis. Wilcoxon sign rank test was used to analyze within group and Kruskal-Wallis ANOVA test was used to analyze between group. All statistical analyses were assessed before surgery and on weeks 2, 4 and 8 after surgery.

## Results

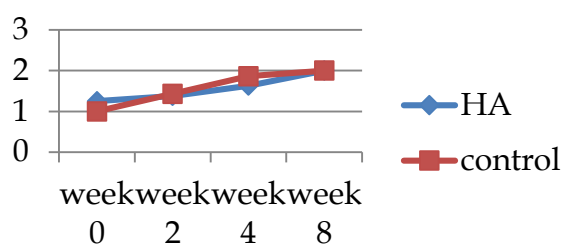
**Descriptive analysis:** The dogs in the HA group were 3 males and 5 females and their average age and weight were  $6.13 \pm 2.23$  years and  $19.75 \pm 11.69$  kilograms, respectively. For the control group, there were 5 males and 3 females and their average age and weight were  $6.29 \pm 1.80$  years and  $16.36 \pm 9.93$  kilograms, respectively. As a result, the variance of age and weight of the two groups was not statistically significantly different ( $p > 0.05$ ).



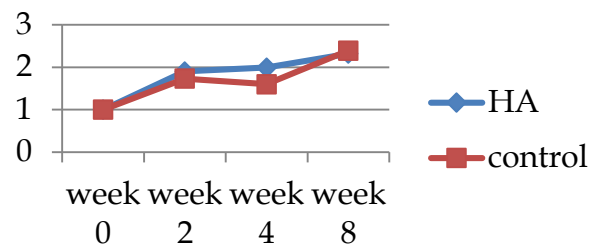
**Figure 1** Lameness between HA and control groups. The x-axis shows study week and the y-axis shows lameness score, with statistically significant difference ( $p < 0.05$ ) in week 2 after surgery.



**Figure 2** Weight bearing between HA and control groups. The x-axis shows study week and the y-axis shows weight bearing score, with statistically significant difference ( $p < 0.05$ ) in week 2 after surgery.



**Figure 3** Radiological score between HA and control groups. The x-axis shows study week and the y-axis shows radiological score, with no statistically significant difference ( $p > 0.05$ ) in every week of the study.



**Figure 4** Level of WF6 in serum between HA and control groups. The x-axis shows study week and the y-axis shows level of serum WF6, with no statistically significant difference ( $p > 0.05$ ) in every week of the study.

**Physical examination:** Physical deformity in clinical examination and hematological disorders of the dogs in both groups were not found since the beginning of the research. Furthermore, all dogs participated in the study until the end.

**Clinical assessment: lameness score:** The levels of lameness of the dogs were continually better; the lameness decreased in both groups of dogs. In the HA group, the level of lameness was statistically significantly different between before operation and 2 weeks after operation, before operation and 4 weeks after operation, before operation and 8 weeks after operation, and 4 weeks after operation and 8 weeks after operation.

In the control group, the level of lameness was statistically significantly different between before operation and 8 weeks after operation, and 2 weeks after operation and 8 weeks after operation. In conclusion, the level of lameness of the HA group was statistically significantly better than that of the control group in week 2 ( $p < 0.05$ ; Fig 1).

**Clinical assessment: weight bearing score:** Similar to the level of lameness, the levels of weight bearing of the dogs in both groups continually increased. In the HA group, the level of weight bearing was statistically significantly different between before operation and 2 weeks after operation, before operation and 4 weeks after operation, and before operation and 8 weeks after operation.

In the control group, the level of weight bearing was statistically significantly different between before operation and 4 weeks after operation, before operation and 8 weeks after operation, and 2 weeks after operation and 8 weeks after operation. In conclusion, the level of weight bearing of the HA group was statistically significantly better than that of the control group in week 2 ( $p < 0.05$ ; Fig 2).

**Radiological assessment:** The scale of radiological images of the dogs in both groups continually increased. As a result, bone regeneration inflated in both groups. In the HA group, the level of radiological images was statistically significantly different between before operation and 8 weeks after operation.

In the control group, the scale of radiological images was statistically significantly different between before operation and 4 weeks after operation, before operation and 8 weeks after operation, and 2 weeks after operation and 8 weeks after operation. However, there was no statistically significant difference between the two groups ( $p > 0.05$ ; Fig 3).

**Biomarker WF6 result:** The level of WF6 in the serum of the dogs in both groups rose during the first 2 weeks after operation whilst the level was rather stable between week 2 and week 4. In week 4 to week 8, however, the level rose again. Comparing between the HA and control groups, the serum in the HA group was slightly higher than that of the control group except for week 8. However, the levels of WF6 in the serum were not statistically significantly different between the groups throughout the study ( $p > 0.05$ ; Fig 4).

## Discussion

Trauma and synovitis with abnormality of stifle joint could cause cruciate ligament disease (Hayashi et al., 2004). Trauma is common in puppies while synovitis with abnormality of stifle joint is often found in older dogs aged 7-10 years (Whitehair et al., 1993). Consequently, correlation of cruciate ligament disease with age was found in this study rather than with gender. Moreover, the weight of dogs commonly found to have cruciate ligament disease is over 22 kilograms (Whitehair et al., 1993), similar to the result of this study.

According to Figure 1, the graph indicates that the intraarticular administration of hyaluronic acid can reduce the pain of stifle joint, in accord with the results of the studies of pain after intraarticular administration of hyaluronan of Gomis et al. (2007), but not with those of Gonzalez-Fuentes et al. (2010) and DeGroot III et al. (2012).

However, because this study was conducted only in dogs in the clinic, there were several uncontrollable factors that could affect the level of lameness including the pain after surgery, the time cruciate ligament disease occurred and the tearing of meniscus before surgery.

In the radiological study, the radiological score of both groups rose in every week. The result conformed to the study of Ghosh et al. (1993) conducted in sheep. According to the study of Ghosh et al. (1993), it was concluded that low molecular weight hyaluronic acid did not affect radiological score, when compared with intraarticular injection of solution, while high molecular weight hyaluronic acid did. Since the molecular weight of hyaluronic acid used in this study was 500-730 kDal, which is considered low, the result of this study agrees with that of Ghosh et al.

The biomarker WF6 between the groups in this study was not statistically significantly different. The risen score in each week of WF6 of both groups in this study was similar to the WF6 score found by Nganvongpanit et al. (2013), who studied the effect of intraarticular administration of hyaluronic acid after joint surgery in dogs. Moreover, the biomarker WF6 within the HA group in week 2 to week 4 after surgery in this study was not statistically significantly different from that in the study of Nganvongpanit et al. (2013). However, the result of this study was in conflict with previous studies which showed that hyaluronan had chondroprotective effect (Corrado et al., 1995; Moreland, 2003; Wang, 2004; Gomis et al., 2007; Strauss et al., 2009).

In this study all dogs did not have any abnormality in complete blood count, serum glutamic pyruvic transaminase enzyme, alkaline phosphatase enzyme, blood urea nitrogen and creatinine, as in previous studies. Joint inflammation that was able to recover within 48 hours was the only side effect found in previous studies (Fernandez Lopez and Ruano-Ravina, 2006; Sun et al., 2006). According to the results of this study and previous studies, it can be summarized that hyaluronic acid is safe for the treatment of osteoarthritis. However, anesthesia may be needed when injecting hyaluronic acid into animals

and multiple injection of hyaluronic acid is suggested for treating osteoarthritis. Therefore, anesthetic risk and further studies of multiple injection of hyaluronic acid should be considered.

All dogs in the control group did not received any intraarticular injection of solution for placebo due to the disadvantage of solution on articular cartilage. Proteoglycan destruction, thickening and fissure of cartilage, and production of some damaging enzyme on cartilage can occur when using solution on cartilage (Bert et al., 1990; Jurvelin et al., 1994; Gradinger et al., 1995; Cheng et al., 2004).

In conclusion, the result from this study shows that hyaluronan has positive effect on lameness and weight bearing after stifle joint surgery. Moreover, it does not affect complete blood count, liver enzyme and kidney function. Nevertheless, intraarticular administration of hyaluronan is ineffective on serum WF6 and radiological exam.

### Acknowledgements

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## บทคัดย่อ

### ประสิทธิภาพของการใช้ไฮยาลูโรนิกแอซิดโดยการบริหารยาเข้าทางข้อต่อภาวะข้อเข่าเสื่อม ภายหลังรับการผ่าตัดรักษาภาวะเอ็นไขว้หน้าหัวเข่าขาดในสุนัข

นที เตชะอารมณ์กุล<sup>1</sup> กัมปนาท สุนทรวิภาต<sup>1\*</sup> ปราชญ์ หมายหาทรัพย์<sup>1</sup>

ภาวะข้อเสื่อมในสุนัข (canine osteoarthritis) ในปัจจุบันสามารถพบได้บ่อยครั้งในคลินิก โดยสาเหตุของภาวะดังกล่าวยังไม่เป็นที่ทราบแน่ชัด การรักษาภาวะข้อเสื่อมในสัตว์โดยมากแล้วเป็นการรักษาเพื่อบรรเทาอาการโดยการให้ยาและผลิตภัณฑ์เภสัชโภชนา ดังนั้นการทดสอบประสิทธิภาพของการใช้ไฮยาลูโรนิกแอซิดและผลิตภัณฑ์เภสัชโภชนาโดยการบริหารยาเข้าทางข้อในการรักษาภาวะข้อเข่าเสื่อมจึงเป็นการศึกษาที่น่าสนใจเพื่อประโยชน์ในการนำมาใช้ทางคลินิก การศึกษานี้ใช้สุนัขที่เข้ารับการผ่าตัดเอ็นไขว้หน้าหัวเข่าขาดจำนวน 16 ตัว โดยแบ่งสุนัขออกเป็น 2 กลุ่ม สุนัขกลุ่มที่หนึ่งได้รับ Hyaluronic acid (Hyalgan<sup>®</sup>) โดยการบริหารผลิตภัณฑ์เข้าทางข้อเข่า ส่วนสุนัขกลุ่มที่สองเป็นกลุ่มควบคุม ใช้คะแนนการกะเผลกของขา การลงน้ำหนักขา ภาพถ่ายทางรังสีวิทยา และระดับตัวชี้วัดทางชีวภาพ WF6 ในการประเมินผลการศึกษา การศึกษาพบว่ากลุ่ม HA มีคะแนนการกะเผลกของขาและการลงน้ำหนักของขาดีกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ส่วนการประเมินด้านอื่น ๆ ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติตลอดระยะเวลาการศึกษา 8 สัปดาห์

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**คำสำคัญ:** เอ็นไขว้หน้าหัวเข่า ไฮยาลูโรนิกแอซิด ผลิตภัณฑ์เภสัชโภชนา ภาวะข้อเสื่อมในสุนัข WF6

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