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A retrospective study of the effect of single dose intra-articular hyaluronic acid injection on postoperative recovery in dogs undergoing stifle surgery

Jiradet Trairatthanom1 Sasithorn Trairatthanom1 Naris Thengchaisri2*

Abstract

Hyaluronic acid (HA) is an abundant molecule found in synovial fluid and plays an important role as a lubricant and shock absorber for the joints. Nonetheless, the effects of synovial fluid replacement with HA after canine stifle surgery remains elusive. A retrospective study was conducted on 54 small breed dogs with either medial patellar luxation (MPL) or cranial cruciate ligament ruptures (CCLR). Dogs were classified as HA (21 with MPL-HA and 16 with CCLR-HA) or control (10 with MPL-C and 7 with CCLR-C) groups. The weight bearing score, lameness score, and pain scores were compared between HA and control groups on 1, 3, 7, 14 and 28 days post-operation. Interestingly, the weight bearing scores at 1 and 7 days post-operation were significantly different between MPL-C and MPL-HA (D1: 4.0±0.0 vs 3.2±0.1, P-value <0.01; D7: 2.8±0.2 vs 2.3±0.1, P-value = 0.038) and between CCLR-C and CCLR-HA (D1: 4.0±0.0 vs 3.7±0.1, P-value = 0.02; D7: 3.2±0.2 vs 2.3±0.1, P-value = 0.02). The lameness score on D28 was significantly different between MPL-C (1.0±0.2) and MPL-HA (0.5±0.1; P-value = 0.045) (Figure 2). However, there was no difference in lameness scores between CCLR-C and CCLR-HA on any assessed day. Pain on palpation was not different between control and HA-treated groups. The present study suggests that synovial fluid replacement with an intra-articular HA injection provides beneficial effects in dogs that have undergone stifle surgery for correction of MPL and CCLR. Optimal frequency of HA administration warrants further study.

Keywords: cruciate ligament, dogs, hyaluronic acid, medial patellar luxation, synovial fluid

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**Introduction**

Synovial fluid functions as a lubricant and shock absorber for human and animal joints (Mow and Wang, 1999; Matthies, 2006), with its main component being hyaluronic acid, allowing for efficient joint movement (Radin et al., 1971). Hyaluronic acid production from the synoviocytes, lining the inner surface of the joint capsule, are substantially reduced due to the inflammatory mediators found in synoviositits. Disease conditions associated with chronic instability of stifle joint including medial patellar luxation (MPL) and cranial cruciate ligament rupture (CCLR) can lead to inflammation and degeneration of the affected joints (Asari et al., 1998). Surgical correction of canine stifle diseases also has an unfavorable effect on joint homeostasis (Dominkun-Chlud, 2011) by altering intra-articular pressure leading to synovial fluid drainage and reduction in synovial fluid viscosity (Kung et al., 2015).

Intra-articular hyaluronic acid injection has been widely used in human (Henrotin et al., 2005) and canine patient (Marshall et al., 2000) with osteoarthritis (Lo et al., 2003; Miller and Block, 2013) by mitigating joint pain and stiffness. Moreover, it is believed that hyaluronic acid helps normalize the properties of joint fluid, reduce cartilage impairment and protect the joint surface (Karay et al., 2004). Amelioration of joint inflammation has been shown in a canine model after intraarticular injection of a gel-like fluid hyaluronic acid after arthroscopy (Pashuck et al., 2016). It has also been used in humans after joint surgery to reduce pain and decrease the postoperative recovery period (Funk and Wykes, 2004; Cohen and Waseem, 2007; Anand et al., 2016).

Interestingly, studies have shown that low molecular weight hyaluronic acid preparation reduced joint inflammation, corrected the properties of synovial fluid, and interrupted cartilage damage (Ghosh and Guidolin, 2002).

Supplementation of HA, a lubricant component of synovial fluid, in dogs that have undergone stifle surgery may improve the postoperative care and decrease the recovery period after surgery. However, there is limited information on the effects of synovial fluid replacement on clinical cases of dogs that have undergone stifle surgery (Nganvorpongani et al., 2013). In the present study, we examine the postoperative effects including the lameness, weight bearing, and pain scores after a single dose of intra-articular injection of low molecular weight hyaluronic acid (500,000-730,000 Daltons) in dogs that had undergone stifle surgery for correction of medial patellar luxation (MPL) or cranial cruciate ligament rupture (CCLR).

**Materials and Methods**

**Study populations:** A retrospective study of 54 small breed dogs undergoing stifle surgery with owner’s consent. The study protocol conformed to the Guide for the Care and Use of Laboratory Animals of Kasetsart University (approval number: ACKU61-VET-031). There were 31 dogs undergoing surgical correction for medial patellar luxation (MPL grade II and III) and there were 23 dogs undergoing surgical correction for cranial cruciate ligament rupture (CCLR) between January 2014 and May 2017. Of the 31 dogs with MPL, 21 dogs received HA (MPL-HA) and 10 received saline (MPL-C). Of the 23 dogs with CCLR, 16 dogs received HA (CCLR-HA) and 7 received saline (CCLR-C). The study recruited only small breed dogs to avoid possible confounding effects caused by different surgical techniques relevant to dog size.

**Preoperative phase:** A physical examination and blood tests (complete blood count, creatinine, alanine aminotransferase and blood parasites) were carried out a day before the surgeries. All dogs were in good condition with no other systemic diseases involved. The dogs in the study were admitted to the hospital for at least 20 hours. Food and water were restricted 12 hours prior to the operations.

**Anesthesia and surgery:** All dogs underwent the same anesthetic protocol. Using a catheter placed into the cephalic vein, intravenous fluids (0.9% NSS or lactated Ringer’s solution) were administered to all dogs. Dogs were premedicated with 0.5 mg/kg of morphine sulfate pentahydrate (FDA of Thailand; M and H manufacturing, Thailand) intramuscularly, and 0.3 mg/kg of diazepam (SIPAM®; Siam pharmaceutical, Thailand) intravenously. Anesthesia was induced with 4 mg/kg of propofol (TROYPOFOL®; Pinyopharmacy, Thailand) intravenously. After endotracheal intubation, the anesthesia was then maintained with isoflurane inhalation (AERRANE®; Baxter, Puerto Rico) and oxygen delivered in a semi-open rebreathing system. Cefazolin sodium (CEFAZOL®; General Drugs House, Thailand) (20 mg/kg, IV) was administered by intravascular injection. Metronidazole (METROGYL®; J.B. Chemicals&Pharmaceuticals, India) (40 mg/kg, IV) was infused intravascularly for 30 minutes.

**Intraoperative phase:** All dogs were operated on by the same surgeon (JT) in the same aseptic environment. Two surgical techniques were used based on the disorder of the dogs. The wedge recession trochleoplasty combined with lateral retinacular imbrication technique (Johnson and Dunning, 2005) was used on dogs with MPL. Dogs with CCLR were operated on using the lateral fabellar suture stabilization combined with lateral retinacular imbrication technique (Johnson and Dunning, 2005). The average surgical time for cranial cruciate ligament rupture and medial patellar luxation was 50±5 and 40±5 min, respectively. A commercially available native HA (HYALGAN®; Fidia Farmaceutici S.P.A., Italy) which contained 20 mg/2 ml sodium hyaluronate with an average molecular weight of 500,000-730,000 Daltons, was used in every dog in the HA treated group. A single dose of 0.5 ml per dog was administered intra-articularly into the affected stifle joint after the lateral retinacular imbrication was completed. Dogs in the control group received only a saline wash before the lateral retinacular imbration and were not given any intra-articular injection.

**Postoperative phase:** After the operation, amoxicillin-clavulanate (CLAVAMOX®, Zoetis, India) at 25 mg/kg, bid (PO), was given to all dogs for 7 days. Carprofen (RIMADYL®, Zoetis, USA) at 2.2 mg/kg, bid
(PO), and tramadol (HIMMADOL®, Seven stars pharmaceutical, Thailand) at 3 mg/kg, bid (PO) were given for post-operative pain control for 7 and 14 days, respectively. Synoquin® (Vetplus, UK) at ¼ tablet of large breed size, sid (PO), was given in all dogs as a joint support supplement for 28 days. Physical therapy was prescribed for every dog. This included 60 repetitions of a passive range of motion exercise followed by ten minutes of cold compression, with an additional ten minutes of warm compression prior to the exercise from D4. This therapy was performed twice a day for 28 days postoperative (D1-D28) to increase movement and flexibility of the joint.

Response to treatment: To measure response to treatment, orthopedic evaluations were conducted on all dogs by the same surgeon throughout the course of the treatment. The assessments were carried out prior to the operation (baseline), and at intervals of 1, 3, 7, 14, and 28 days after the operations. Each dog was evaluated for lameness, weight bearing, and pain on joint movement and assigned scores of 0-4 according to their clinical signs while standing, walking and during joint palpation (Knap et al., 2007). The details of the scoring systems for lameness, weight bearing and pain on palpation are shown in Table 1 (Knap et al., 2007).

Table 1 Descriptions of lameness, weight bearing, and pain scores for assessing the outcome of treatment at pre- and post-operation periods.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lameness scores</td>
<td>0</td>
<td>No sign of lameness, able to walk without any difficulty</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>A mild lameness, walking with shorter strides and lameness is noted when turning or shifting leg</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>A moderate lameness, weight bearing lameness at walk with distinct “head bob”</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>A severe lameness, a toe-touching lameness at walk</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Non weight bearing lameness</td>
</tr>
<tr>
<td>Weight bearing scores</td>
<td>0</td>
<td>Normal weight bearing at standing position and at walk</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Bears weight normally at standing position but places partial weight at walk</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Bears weight partially at standing position and places partial weight at walk</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>A toe-touching weight bearing, dog placed partial weight when standing and holding up the leg at walk</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Non-weight-bearing, holds up the leg at standing position and does not walk</td>
</tr>
<tr>
<td>Pain on palpation scores</td>
<td>0</td>
<td>No signs of pain</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Shows signs of mild pain, dog allows for joint palpation, but pulls back the leg during passive range of motion testing</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Show signs of moderate pain, dog reacts (ie. cries, flinches, pulls away leg) during joint palpation and during passive range of motion testing</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Show signs of severe pain, dog has dramatic response (cries, groan, bites) during joint palpation and struggles (cry, growl, bite) for passive range of motion testing</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Groans and screams continuously at rest, dog refuses for a joint palpation and passive range of motion testing</td>
</tr>
</tbody>
</table>

Data collection: Data related to characteristics of the dogs (i.e., gender, breed, age, and body weight), diseases (MPL or CCLR), side of affected joint, and severity grade of MPL were obtained from the hospital records. Data collections, before the operation (baseline) and at intervals of 1, 3, 7, 14, and 28 days after the operations, were performed by the surgeon (JT).

Statistical analyses: Descriptive statistics were used to describe the dog characteristics and response to treatment outcomes. Univariate statistics were computed for continuous variables (i.e. age, body weight, and measurements of response to treatment), and categorical variables (i.e., gender, and breed) were described with a frequency distribution for each disease and treatment group (HA treated and control).

A comparison of response to treatment outcomes between the HA treated and control groups was carried out for each different assessment time. Considering the difference in disease mechanisms of MPL and CCLR, which may have resulted in different levels of pain and lameness, we ran the analyses separately for each orthopedic condition. Other factors (e.g., body weight, age, gender, and side of affected limb) were also tested for potential confounding effects, and it appeared that the data in the two treatment groups was comparable and, therefore the analyses did not require adjustment for these confounding factors. The analysis was performed using t-test with unequal variance. P-value of less than or equal to 0.05 was considered statistical significance.

Results

Of all the 54 dogs that underwent stifle surgery, 31 dogs presented with MPL (31±3 months) were significantly younger than the 23 dogs presented with CCLR (67±3 months; P-value<0.01). However, there was no significant difference in age between the control and HA-treated groups in dogs presented with MPL (means of MPL-C group: 25±4; MPL-HA group: 34±4, P-value = 0.09) or dogs presented with CCLR (means of CCLR-C group: 64±5; CCLR-HA group: 68±4, P-value = 0.55). Other characteristics of the dogs in each group are presented in Table 2. No significant difference in dog gender within the control and HA treated groups of dogs presented with MPL (P-value = 0.31) and dogs presented with CCLR (P-value = 0.47).

Comparisons of the weight bearing scores at different assessment times is shown in Figure 1. At the preoperative time (D0), there was no significant difference in preoperative weight bearing between MPL-C (0.4±0.2) and MPL-HA (0.3±0.1, P-value = 0.74) or between CCLR-C (2.7±0.3) and CCLR-HA (2.8±0.2, P-value = 0.77) (Figure 1). Interestingly, the weight
bearing scores of on D1 and D7 was significantly different between MPL-C and MPL-HA (D1: 4.0±0.0 vs 3.2±0.1, P-value <0.01; D7: 2.8±0.2 vs 2.3±0.1, P-value = 0.038) and between CCLR-C and CCLR-HA (D1: 4.0±0.0 vs 3.7±0.1, P-value = 0.02; D7: 3.2±0.2 vs 2.3±0.1, P-value = 0.02) (Figure 1).

Assessment of lameness is shown in Figure 2. At the preoperative time (D0), there was no significant difference in preoperative lameness scores between MPL-C (0.4±0.2) and MPL-HA (0.3±0.1, P-value = 0.74) or between CCLR-C (3.3±0.2) and CCLR-HA (3.3±0.1, P-value = 0.87) (Figure 2). Moreover, the lameness score on D28 was significantly different between MPL-C (1.0±0.2) and MPL-HA (0.5±0.1; P-value = 0.045) (Figure 2). However, there was no difference in lameness scores between CCLR-C and CCLR-HA throughout the study periods.

The outcome of HA on pain reduction was examined during limb palpation (Figure 3). At the preoperative time (D0), there was no significant difference in preoperative pain on palpation scores between MPL-C (0.2±0.1) and MPL-HA (0.1±0.1, P-value = 0.71) or between CCLR-C (2.1±0.1) and CCLR-HA (2.2±0.1, P-value = 0.80) (Figure 3). Moreover, there was no difference in pain on palpation scores between the control and HA-treated groups in dogs with MPL or CCLR on any of the assessed days (Figure 3).

Table 2  General characteristics of dogs with medial patellar luxation (MPL) or cranial cruciate ligament rupture (CCLR). Intraarticular hyaluronic acid (HA) injection treatment status is indicated by the letters -C (control) and -HA (HA treated).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MPL-C</th>
<th>MPL-HA</th>
<th>CCLR-C</th>
<th>CCLR-HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10</td>
<td>21</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>12</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>9</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Age in month</td>
<td>25±12</td>
<td>34±18</td>
<td>64±13</td>
<td>68±18</td>
</tr>
<tr>
<td>(minimum, maximum)</td>
<td>(12, 52)</td>
<td>(10, 72)</td>
<td>(44, 84)</td>
<td>(30, 96)</td>
</tr>
<tr>
<td>Body weight in kg</td>
<td>3.6 (1.1)</td>
<td>3.6 (1.1)</td>
<td>4.1 (0.8)</td>
<td>4.6 (1.2)</td>
</tr>
<tr>
<td>(minimum, maximum)</td>
<td>(2.3, 6.2)</td>
<td>(1.9, 6.2)</td>
<td>(3.2, 5.6)</td>
<td>(2.8, 7.0)</td>
</tr>
</tbody>
</table>

Figure 1  Weight bearing scores (mean and 95% confidence interval) of dogs receiving a single dose intra-articular hyaluronic acid vs control groups by orthopedic conditions: (A) MPL - medial patellar luxation (circle) and (B) CCLR - cranial cruciate ligament rupture (triangle). The open symbol refers to dogs in the control group (C), and opaque symbol designates dogs that received a single dose intra-articular hyaluronic acid (HA) on the day of operation. An asterisk (*) indicates statistical significance P-value<0.05.
Figure 2  Lameness scores (mean and 95% confidence interval) of dogs receiving a single dose intra-articular hyaluronic acid vs control groups by orthopedic conditions: (A) MPL – medial patellar luxation (circle) and (B) CCLR – cranial cruciate ligament rupture (triangle). The open symbol refers to dogs in the control group (C), and opaque symbol designates dogs that received a single dose intra-articular hyaluronic acid (HA) on the day of operation. An asterisk (*) indicates statistical significance $P$-value<0.05.

Figure 3  Pain on palpation scores (mean and 95% confidence interval) of dogs receiving a single dose intra-articular hyaluronic acid vs control groups by orthopedic conditions: (A) MPL – medial patellar luxation (circle) and (B) CCLR – cranial cruciate ligament rupture (triangle). The open symbol refers to dogs in the control group (C), and opaque symbol designates dogs that received a single dose intra-articular hyaluronic acid (HA) on the day of operation.
Discussion

A single dose intra-articular hyaluronic acid injection appeared to have a positive effect on the weight bearing of dogs with MPL only on post-operation days 1 and 7 (Figure 1). After that, all dogs showed similar weight bearing scores regardless of whether the HA treatment has been applied. This maybe due to the initial effects of lubricative and anti-inflammatory properties of HA that helped HA-treated dogs to overcome pain, allowing them to move the joint and bear weight on the affected limb. However, the beneficial effect of HA did not last long due to the short intrasynovial half-life (less than 24 hour) (Brown et al., 1991; Fraser et al., 1993; Lindenhayn et al., 1997) and rapid degradation of HA (1-3 days for 1-2% HA of 50,000-6,000,000 Daltons) (Brown et al., 1991; Burdick et al., 2005).

Osteoarthritis is a progressive disorder affecting the synovial joint characterized by degeneration and erosion of the joint cartilage leading to variable degrees of synovitis and ostophyte formation of the surrounding bone (Johnston, 1997). Two main types of osteoarthritis are commonly classified. Primary osteoarthritis is associated with aging and wear of the articular cartilage that can affect various joints of the body such as elbow, hip, stifte, and spine (Pettitt and German, 2015). Secondary osteoarthritis has many acquired and congenital causes including ligament rupture (CCLR) abnormal skeletal conformation (MPL) and osteochondrosis of the shoulder causing an instability and alteration of the joint biomechanics. For instance, cranioaudal translation of the stifte joint is primarily maintained by cranial cruciate ligament. Dogs with CCLR leads to anterior subluxation of the tibia leading to an impactation of posterior aspect of tibial plateau against anterior aspect of lateral femoral condyle (Simon et al., 2015) on the weight bearing area contributing to progressive osteoarthritic changes, pain and limb dysfunction. In contrast, MPL is a developmental condition due to the loss of normal tracking of patellar in the femoral trochlea leading to kneecap luxation (Alam et al., 2011). The loss of stabilization force from quadriceps muscle leads to bone deformity of the femur and tibia that worsen with growth. The degenerative change of the articular cartilage in MPL is due traction and rubbing of the unstable kneecap causing the cartilage to slowly wear at a slower pace when compared with CCLR. The changes of the collagen network as well as a decrease of proteoglycan leads to a softening of the articular cartilage (Alam et al., 2011). Degeneration of the articular cartilage facilitates the ablation of the cartilage down to the subcondral bone leading to inflammation and progression of osteoarthritis. A significant reduction in the viscosity of synovial fluid is obtained from joints with osteoarthritis, therefore administration of hyaluronic acid may help improve synovial fluid viscosity, promote cartilage healing as well as reduce pain and inflammation (Carapeba et al., 2016). The results of the present study supported a beneficial effect of HA injection in dogs undergoing surgical repair for CCLR and patellar luxation.

A previous study (Nganvongpanit et al., 2013) did not find any difference in lameness, weight bearing, and pain scores between the control and the dogs treated with a single dose intra-articular HA injection in dogs with MPL for the 1-4 weeks follow-up. However, our findings revealed an early effect of HA injection on weight bearing within the first postoperative day (Figure 1). The discrepancy between the results may be partly due to the difference in assessment intervals between the studies. Nganvongpanit et al. (2013) began the first postoperative evaluation a week later, thus HA may have already been degraded and eliminated from the joint. Similarly, a study of equine lameness did not show a significant effect of a single dose intra-articular HA injection in horses with metacarpophalangeal joint problems, evaluated on Day 14 after treatment (Niemelä et al., 2016). Hence, any short-term effect that HA may have had was not detected. Thus, the present study supported the early effect of HA injection for dogs undergoing stifte surgery.

The lameness score in the MPL-HA group was significantly lower than MPL-C (Figure 2), suggesting the beneficial effect of HA injection over a late effect of HA injection on joint recovery. However, there was no significant difference in lameness scores between CCLR-C and CCLR-HA groups (Figure 2). The different outcome may be due to components of chronic inflammation associated with CCLR. Thus, a single dose of intra-articular HA may not be sufficient for a treatment of chronic joint inflammation in CCLR. It is possible that assessment using the lameness score in the present study may not be sensitive enough to demonstrate the efficacy of the HA treatment. An alternative approach to determine the response to treatment is to use a quantitative method such as canine force plate gait analysis (Vijarnsorn, 2002). This approach provides detailed information, such as the stride length and the peak vertical force of each stride quantified in numbers, and has been used in several studies (Budsberg et al., 1987; Brown et al., 2013; Kwananocha et al., 2016).

Different preparation of HA contributes to different therapeutic outcome of HA for arthritis synovium (Asari et al., 1998). It has been shown that HA with a molecular weight (MW) between 500,000 to 1,000,000 Da was more effective than HA with a MW more than 2,500,000 Da. A short-term study indicated that the smaller HA molecule is more approachable for synovial lining cells leading to better prevention of joint degeneration by inhibiting inflammation and PGF2 release (Asari et al., 1998). Palpation of the joint was used to assess for signs of inflammation in the present study. Nonetheless, the present study did not identify a beneficial effect of HA injection (MW 500,000-730,000 Daltons) for pain reduction after surgical correction for CCLR or MPL (Figure 3). Interestingly, a long-term study revealed similar improvement of joint stiffness using either intra-articular injection of low or high MW HA for knee osteoarthritis (Gigis et al., 2016). Nonetheless, both low and high MW HA failed to slow down the radiographic progression of knee osteoarthritis (Gigis et al., 2016).

In conclusion, the present study suggests that synovial fluid replacement with an intra-articular HA injection is helpful for dogs that have undergone stifte
surgery to regain the early use of the affected limb. Thus, intra-articular HA injection can be considered as an additional treatment during canine stifle surgery. Further studies should be done to evaluate the effects of multiple doses of intra-articular HA and determine the optimal frequency of administration to improve the clinical outcome in dogs that undergo stifle surgery.

Acknowledgements

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