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Spontaneous Osteoarthritis in Dogs - Clinical Effects of Single and Multiple Intra-articular Injections of Hyaluronic Acid

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ABSTRACT

***Background*:** The treatments of osteoarthritis (OA) are commonly conservative and multimodal to relieve pain and improve movement. Intra-articular injection of hyaluronic acid (IAHA) has been studied as a treatment option for OA in dogs. IAHA helps restore the viscoelasticity of the synovial fluid and relieves the clinical symptoms of OA. However, the efficacy of IAHA in dogs is still a controversial subject. This study aims to confirm the IAHA effect in dogs with spontaneous OA and to compare the effectiveness depending on the number of injections.

***Materials, Methods & Results*:** Thirty dogs with spontaneous OA were assigned to a single injection group (n=17) and a 3-weekly injections group (n=13). Dogs weighing less than 10 kg were injected 1 mL of HA to the OA joint, and more than 10 kg dogs were injected 2 mL of HA. In the case of the 3-weekly injections group, the same amount was administered 3 times at 1-week intervals. After the injection, physical and orthopedic examinations were performed to check for complications. Radiographic OA score was evaluated before and 3 months after the injection to confirm and to evaluate the progression of OA. Clinical symptom evaluations were performed on pre-injection, 1-, 2-, and 3-months post-injection. They consisted of the clinical lameness score by veterinarians and Canine Brief Pain Inventory (CBPI) by owners. Results were compared with unpaired t-test, repeated-measures ANOVA with Tukey's or Sidak's multiple comparison test, or Wilcoxon test, with $P < 0.05$. Patients had a median age of 9 years (range 3 to 16 years) and a bodyweight of 4.8 kg (range 2 to 48 kg). No systemic side effects or major complications were detected during the trial period. IAHA produced temporary pain and discomfort in 6 cases. There was no change in the radiographic OA score before and 3 months after injections in both groups, and the difference between groups was not confirmed. In both groups, the clinical lameness score significantly decreased at 1, 2, 3 months after injection compared with pre-injection. The score was lower at 3 months after the injection than at 1 month. The clinical lameness score had no significant difference between the groups. Similarly, CBPI was all decreased in the single injection group and 3-weekly injections group compared to pre-injection, and the score at 3 months post-injection was lower than at 1 month. No significant differences between the groups were found in CBPI.

***Discussion*:** Most studies on the efficacy of IAHA in canine OA have been conducted using an experimental model, so studies on spontaneous canine OA are insufficient. This study confirmed that IAHA improves clinical symptoms such as pain relief and movement improvement in spontaneous OA dogs using CBPI and clinical lameness score. In order to confirm the optimal IAHA protocol, a single IAHA and 3-weekly IAHA were compared. The result shows that clinical symptoms improved in both single and 3-weekly injections groups, but no significant difference was confirmed during the 3-month study period. These findings may suggest that a single IAHA may have a similar effect to multiple IAHA, and repeated injections are unnecessary. In humans and canine OA models, it is reported that the effect of IAHA was maintained for 6 months. This study showed that the effect of IAHA was maintained for 3 months study period and that clinical symptoms improved at 3 months than at 1 month. In conclusion, these findings suggested that IAHA improves clinical symptoms in dogs with spontaneous OA, and a single IAHA showed a similar effect to 3 weekly IAHA.

Keywords: canine, treatment, hyaluronic acid, intra-articular injection, osteoarthritis

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INTRODUCTION

The goal of osteoarthritis (OA) management is to alleviate pain, slow the progression of cartilage damage, increase joint flexibility and, ultimately, improve quality of life [2,5,22]. Although nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed medication for OA, long-term use may have negative effects on the gastrointestinal tract, kidneys, and liver [10,13]. As a result, complementary and alternative medicine has increasingly been offered as an additional OA treatment option.

Hyaluronic acid (HA) is a hydrophilic glycosaminoglycan found in synovial fluid and cartilage matrix. HA imparts viscoelasticity and lubricity to synovial fluid, thereby smoothing joint movement and absorbing shock [9,14]. The molecular weight and concentration of HA in osteoarthritis synovial fluid decrease due to dilution, fragmentation, and abnormal production by synoviocytes [11]. As a result, the viscoelasticity of the synovial fluid is reduced, and the joint's homeostasis is disrupted. Intra-articular injection of HA (IAHA) helps restore synovial fluid viscoelasticity and alleviate the clinical symptoms of OA [22].

However, the efficacy of IAHA in spontaneous canine OA is still a controversial subject [2,12,23]. Furthermore, previous studies have reported on the safety and efficacy of HA in the treatment of canine OA, but most of them used the experimental OA model [16,18,21,27]. This study aims to determine whether IAHA helps improve clinical symptoms in dogs with naturally occurring OA and to compare the clinical symptom improvement effects of a single HA injection versus 3 weekly HA injections.

MATERIALS AND METHODS

Study design

The study was planned as a multicentre, prospective, and open-label clinical trial.

Inclusion criteria

This study included 30 dogs with clinical symptoms of OA in the stifle or hip joints. All of the dogs were client-owned and came to the veterinary hospitals to be treated for clinical symptoms of OA. Dogs were chosen for this trial if they were more than 1-year-old, weighed > 2.5 kg, had clinical symptoms that lasted more than 1 month and had radiologic abnormalities. If a dog had OA in more than one joint, the most severely affected joint was evaluated to determine the effectiveness of the treatment. The exclusion criteria were as follows: underlying disease that could affect the study's outcome, lameness caused by neurological issues, neoplasia or immune-mediated disease and oral medication administration or intra-articular injection within 2 weeks before and during the trial.

Administration of hyaluronic acid

The dogs were divided into 2 groups: single injections and 3 injections. Seventeen dogs were assigned to the single injection group and 13 to the 3 injections group. Following aseptically preparing the injection site, arthrocentesis was performed with a 23-gauge needle and a 3 mL syringe. To ensure that the needle was properly positioned in the joint, synovial fluid was withdrawn before injecting HA. For less than 10 kg dogs, 1 mL of HA[1] was injected, while dogs weighing more than 10 kg received 2 mL of hyaluronic acid. In the 3 injections group, 3 IAHA were administered at weekly intervals.

Radiographic evaluation

At the start of the study and 3 months after the IAHA, radiographs of the affected joint were taken. The radiographic images were evaluated for osteophytes, subchondral sclerosis, and bone remodelling (Table 1) to determine the severity of OA [4].

Table 1. Osteoarthritis severity radiographic scoring scale.

Score	Clinical evaluation
0	Normal joint
1	Radiographic evidence of instability; no degenerative change
2	Mild degenerative change (occasional osteophytes)
3	Moderate degenerative change (osteophytes, subchondral sclerosis)
4	Severe degenerative change (osteophytes, subchondral sclerosis, bone remodelling)

Veterinary assessment

Veterinarians performed a basic physical examination, palpation and visual evaluation of the injection site and orthopaedic examination 3 days after IAHA to check for complications. Before, 1, 2, and 3 months after the IAHA, orthopedic examinations were performed. Standing posture, lameness, weight-bearing, range of motion, pain at mobilization and overall clinical condition were assessed and scored by veterinarians using the previously described criteria (Table 2) [16].

Table 2. The criteria for evaluating clinical sign and activity in osteoarthritis dog (vet score).

Score	Criteria
	V-Q1 Standing posture
1	Normal stance
2	Slightly abnormal stance (partial weight-bearing of the limb, but the paw remains firmly in contact with floor)
3	Markedly abnormal stance (partial weight-bearing of the limb, with minimal contact between the paw and floor)
4	Severely abnormal stance (no weight-bearing)
	V-Q2 Lameness at walk
1	No lameness; normal weight-bearing on all strides observed
2	Mild lameness with partial weight-bearing
3	Obvious lameness with partial weight-bearing
4	Marked lameness with no weight-bearing
	V-Q3 Lameness at trot
1	No lameness; normal weight-bearing on all strides observed
2	Mild lameness with partial weight-bearing
3	Obvious lameness with partial weight-bearing
4	Marked lameness with no weight-bearing
	V-Q4 Willingness to allow the clinician to lift the limb contralateral to the affected limb
1	Readily accepts contralateral limb elevation, bears full weight on the affected limb for more than 30 s
2	Offers mild resistance to contralateral limb elevation, bears full weight on the affected limb for more than 30 s
3	Offers moderate resistance to contralateral limb elevation and replaces it in less than 30 s
4	Offers strong resistance to elevation of contralateral limb and replaces it in less than 10 s
5	Refuses to raise contralateral limb
	V-Q5 Range of motion (ROM)
1	Full ROM
2	Mild decrease (10%-20%), with no crepitus
3	Mild decrease (10%-20%), with crepitus
4	Moderate decrease (20%-50%)
5	Severe decrease (≥ 50%)
	V-Q6 Pain at palpation/mobilisation
1	No pain elicited on palpation/mobilisation of the affected joint
2	Mild pain elicited, e.g. turns the head in recognition
3	Moderate pain elicited, e.g. pulls the limb away
4	Severe pain elicited, e.g. vocalises or becomes aggressive
5	Severe pain elicited, e.g. not allow examiner to palpate/mobilise the joint
	V-Q7 Evaluation of overall clinical condition
1	Good
2	Mildly poor
3	Moderately poor
4	Severely poor
5	Very severely poor

Owner assessment

During the orthopaedic examination, owners used the Canine Brief Pain Inventory (CBPI) to assess the condition of their dogs. The CBPI, which consists of pain severity scores (PSS) and pain interference scores (PIS), can quantify the severity of chronic pain in dogs with OA [6,7]. To confirm clinical improvement, the PSS and PIS values, as well as the sum of these, CBPI scores were compared. Lower scores denote less severe pain.

Statistical analysis

GraphPad Prism[2] was used for statistical analysis, and a $P < 0.05$ was considered significant. Each graph's data and bar represent the mean value ± standard deviation (SD).

To confirm appropriate population assignment, the unpaired *t*-test was used to compare weight, age and baseline of veterinary assessment and owner assessment between groups.

The Wilcoxon test was used to compare differences in radiographic OA scores before and after injection in all dogs and groups. To compare radiographic scores between groups at each period, repeated measure analysis of variance (ANOVA) with Tukey's multiple comparison test was used.

To determine the significance of differences within groups over time, repeated measure ANOVA with Tukey's multiple comparison test was used for orthopaedic examination and owner assessment. To compare groups at each time point, a Two-way ANOVA with Sidak's multiple comparison test was used.

RESULTS

Animals

The clinical trial included 30 dogs, and information about the dogs is summarised in Table 3. Dogs ranged in age from 3 to 16 years old (median 9 years old), with weights ranging from 2 to 48 kg (median 4.8 kg). All dogs had a mean vet score of 16.70 and a mean CBPI score of 126.30. There were no significant differences in weight, age, the baseline of vet score and CBPI between the single and 3 injections groups.

Radiographic evaluation

For all dogs, radiographic OA scores at 3 months post-injection were not significantly different from pre-injection. There was no significant difference in OA scores between the single and 3 injections groups. The OA scores were not significantly different between groups at each time point (Figure 1).

Veterinary assessment

After the IAHA, no systemic complications, injection site swelling, redness, or heat were found. However, 6 dogs (6/30 = 20%) experienced discomfort and pain for 2-3 days following injection. In all 6 dogs, the discomfort and pain subsided within 3 days without additional treatment. There was no significant difference between the single (3/17 = 17.6%) and 3 injections groups (3/13 = 23%) in the veterinary assessment outcomes ($P > 0.05$).

The vet scores for each index were summed and evaluated to compare before and 1, 2, and 3 months after intra-articular injection. When all patients were analysed, the vet scores at post-injection were significantly lower than at pre-injection, and the scores at 2 and 3 months post-injection were lower than at 1 month post-injection ($P < 0.05$). Vet scores significantly decreased after the IAHA in both the single and 3 injections groups, and the vet score at 3 months post-injection was lower than at 1 month post-injection ($P < 0.05$) [Figure 2]. Over time, there was no significant difference between the single and 3 injections groups ($P > 0.05$).

Owner assessment

When the owner assessment for all patients was evaluated, PIS, PSS, and CBPI decreased 1 month after injection compared to pre-injection scores, and the scores were lower at 3 months after injection than at 1 month after injection ($P < 0.05$). Similarly, in the single and 3 injections groups, PIS, PSS and CBPI were all lower 2 months after IAHA ($P < 0.05$). There was no significant difference in CBPI, PSS or PIS between the 2 groups at any time point ($P > 0.05$) [Figure 3].

Table 3. Characteristics of clinical trial population of dogs with clinical symptoms of osteoarthritis (OA) in the stifle or hip joints.

	Patient data		
	One injection (n = 17)	Three injections (n = 13)	*P*-value
Body weight (kg, mean ± SD)	7.87 ± 2.8	8.87 ± 3.4	0.82
Age (years, mean ± SD)	9.52 ± 0.84	9.3 ± 1.21	0.88
Male/female	6/11	5/8	
Affected joint (n/total)			
Left stifle joint	10/17	9/13	
Right stifle joint	6/17	4/13	
Left coxofemoral joint	1/17		
Breed (n/total)			
Poodle	2/17		
Pomeranian	2/17	1/13	
Yorkshire Terrier	4/17	2/13	
Mixed	5/17	1/13	
Cocker Spaniel	1/17		
Shih Tzu	1/17		
Maltese	1/17	6/13	
Shetland Sheepdog		1/13	
Spitz		1/13	
Malamute	1/17	1/13	
CBPI† (baseline, mean ± SD)	137.2 ± 9.58	112 ± 13.49	0.13
Vet score (baseline, mean ± SD)	17 ± 1.29	16.31 ± 1.23	0.7

†CBPI= Canine Brief Pain Inventory score.

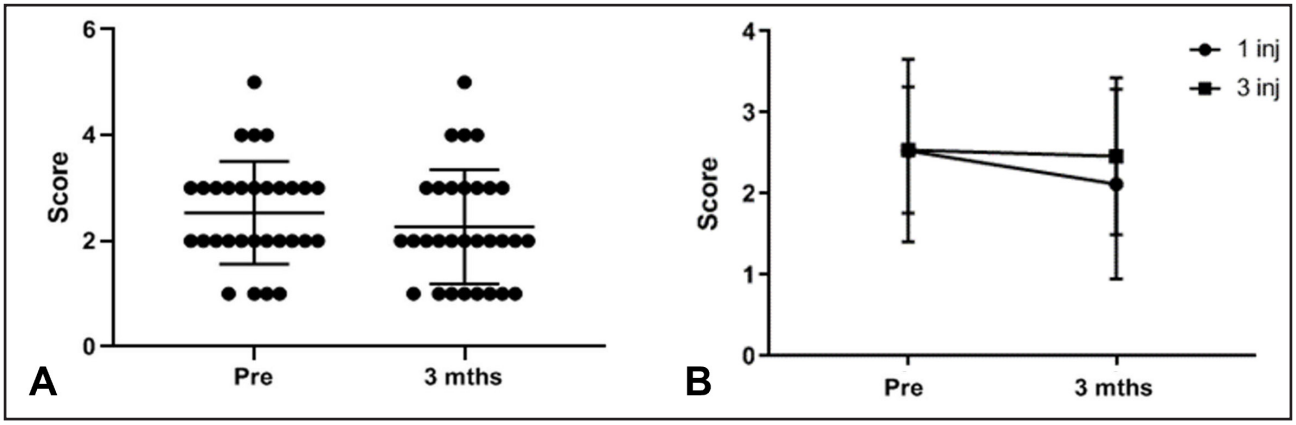

Figure 1. Radiographic OA score evaluation. A- Comparison of OA score before and 3 months after injection in all dogs. B- Comparison of OA scores between single and three injections groups at each time point. No significant differences compared to before injection and between the group.

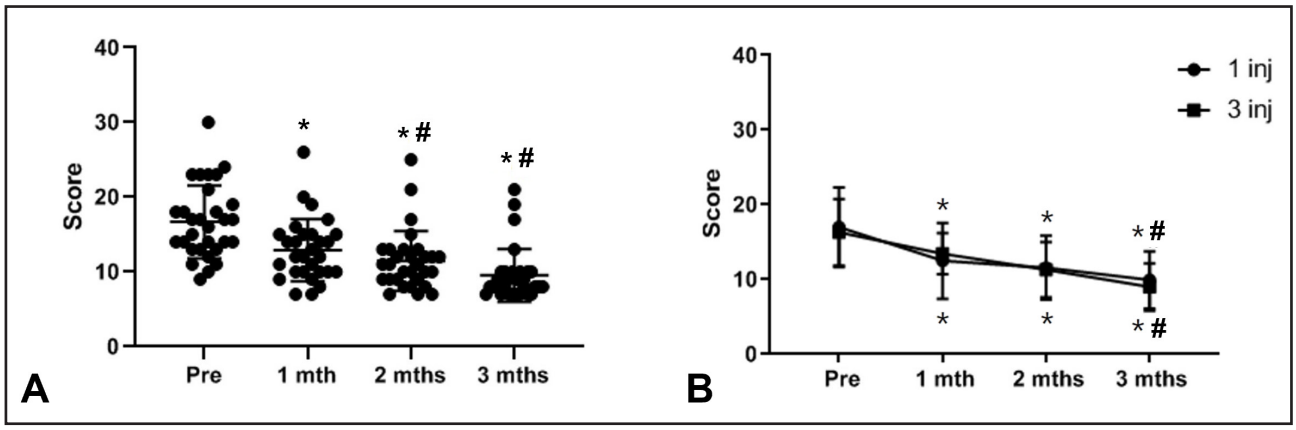

Figure 2. Vet score assessment. A- In all dogs, the sum of vet scores was compared over time before and after injection. B- Comparison of vet score summation over time in each group and between groups.*Significant difference compared to before injection ($P < 0.05$). #Significant difference compared to after 1 month ($P < 0.05$). No significant differences between groups were identified.

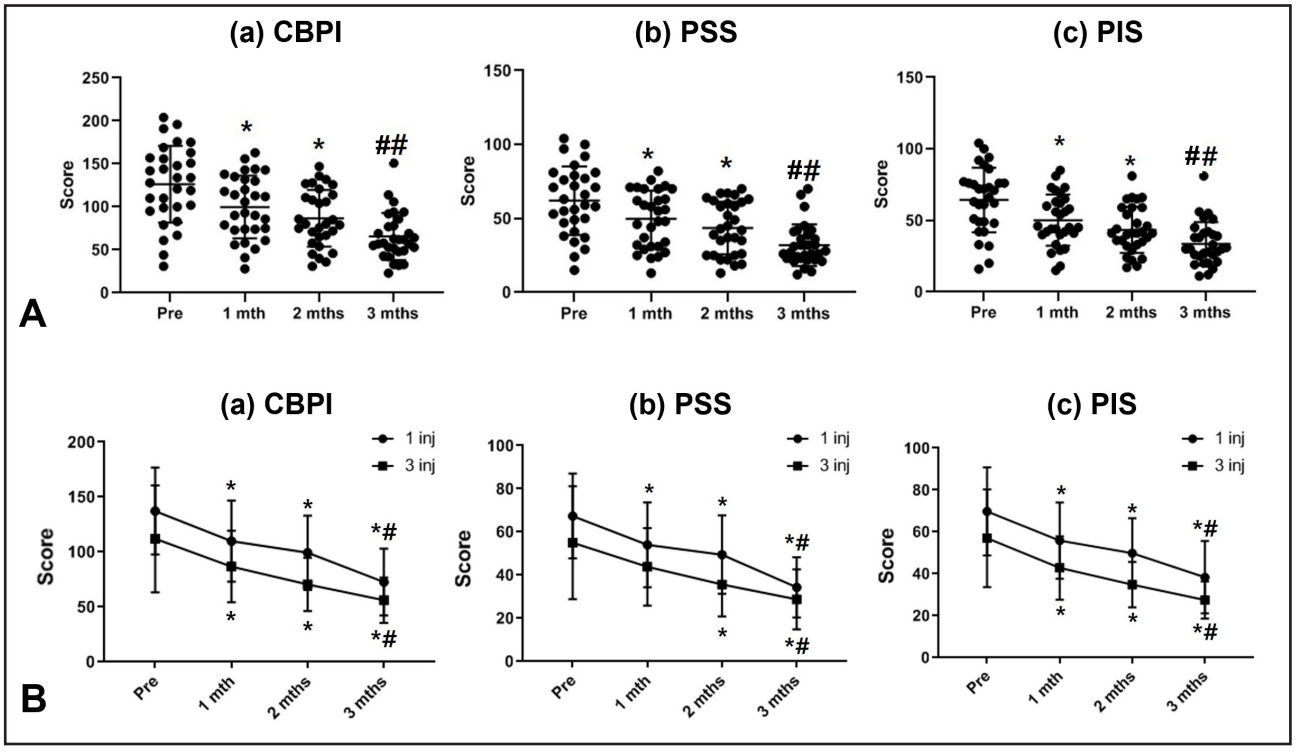

Figure 3. Canine Brief Pain Index (CBPI, a) assessment consisting of pain severity score (PSS, b) and pain interference scores (PIS, c). A- Comparison of time-specific differences in all patients. B- Comparison of time-specific differences in each group and group-to-group differences. *Significant difference compared to before injection ($P < 0.05$). #Significant difference compared to after 1 month ($P < 0.05$). No significant differences between groups.

DISCUSSION

This study aimed to confirm the effects of a single and 3 weekly IAHA on spontaneous canine OA using subjective measures of lameness severity (vet score) and pain (CBPI). When analyzing all dogs with IAHA, the results showed that all assessment parameters significantly improved throughout the 3-month post-injection. These findings provide empirical evidence that IAHA relieves pain and assists functional recovery in canine OA.

This study was carried out on client-owned dogs suffering from spontaneous OA. Most of the previous studies on the efficacy of IAHA in canine OA have been conducted using an experimental OA model

induced by transecting the cranial cruciate ligament [16,18,21]. The pathology of surgically induced OA differs from that of naturally occurring OA [15,17], and the symptoms and progression of OA greatly vary from dog to dog, necessitating research into naturally occurring OA. Few studies have been published on the efficacy of IAHA in client-owned dogs with hip dysplasia [1,24] and undergoing joint surgery [20,26]. In contrast to previous studies, this study was performed IAHA primarily on the stifle joint (29/30 joints) in dogs undergoing conservative treatment for OA. These findings, along with previous research, suggest that IAHA may be a viable treatment option for spontaneous canine OA.

The efficacy of IAHA was tracked for 3 months in the present study. The effects of IAHA in humans and canine OA models have been reported to last for approximately 6 months [21,25]. To accurately determine IAHA duration, a longer evaluation period is required. However, since the beneficial effect of HA has been reported to be remarkable at 5-13 weeks [28] and peaks at 4-8 weeks [3,21], 3 months was deemed adequate to evaluate the effect of IAHA. Furthermore, the fact that the efficacy of IAHA was maintained for 3 months and improved more than at 1 month at 3 months suggests that the efficacy of IAHA can be sustained for at least 3 months and may last for a longer period.

There are no consensus protocols for IAHA in veterinary medicine. Prior research on IAHA in canine OA has found that single and multiple injections at weekly intervals are effective [18,21,24], but it has not been determined which HA injection dosing regimen is superior. In human medicine, 3-5 times IAHA at weekly intervals for low-molecular-weight (MW) HA and a single high dose for high-molecular-weight HA are commonly recommended [25]. A human knee OA study found that a single larger dose of intermediate MW HA is as effective as repeated doses and is more economical [25]. In order to confirm the optimal IAHA protocol, single intra-articular injection and 3 intra-articular injections at a weekly interval were performed in this study, as is commonly recommended when using intermediate MW HA. Single intra-articular injections may be a viable injection option if the difference between single and multiple effects is not significant. During the 3-month study period, no significant difference in subjective assessment using CBPI and vet score was observed between the single and 3 injections groups. Both groups gradually recovered up to the third month, with post-injection scores significantly higher than pre-injection. In the canine OA research, single and 2 IAHA after patella luxation repair surgery showed similar amelioration effects [20]. In the experimental model, no significant difference in the effects of single and 3 weekly high-molecular-weight HA injections was found [21]. Having the same effect as multiple doses with a single injection may mean that repeated administration is unnecessary. Repeated injections may increase the risk of infection, treatment cost and patient stress and repeated sedation is required because sedation is recommended during the intra-articular injection. The findings of the current study may suggest that a single IAHA may have the same effect as multiple injections and repeated injections would not be required. Furthermore, a single IAHA may be a feasible and convenient treatment method.

Transient pain and discomfort were noted following injection in some dogs (6 of 30 dogs) and all these events naturally resolved within 3 days without treatment. During the study period, no dogs experienced systemic problems or serious adverse events such as septic arthritis. This finding is consistent with previous research, which found that IAHA in dogs could cause minor side effects such as transient mild pain, redness, heat and swelling but did not result in serious complications [8,19,20]. In this study, no correlation was found between the number of injections and the local response. Both single and 3 weekly IAHA in canine OA are well-tolerated and relatively safe methods. Furthermore, IAHA may be an effective OA treatment option in dogs for whom NSAIDs or other analgesics are contraindicated.

This study has some limitations since it was conducted only with subjective methods (CBPI and vet score), and objective evaluation like kinetic gait analysis was not performed. However, the CBPI used in this study is known as a way to confirm the effect of OA treatment and is a widely used method [6,7]. In addition, for more precise evaluation, vet scores were evaluated using the modified clinical lameness score system, including subdivided evaluation items. Another limitation is that this study was conducted for a short period. If the evaluation period was extended, the duration of the single and multiple IAHA could be checked and compared. As a result, further research with a larger

population and over a longer time is required to expand on the findings of this study.

CONCLUSION

In conclusion, this study demonstrate that IAHA relieves pain and enhance functional movement in dogs with naturally occurring OA through CBPI and veterinary assessment, and a single IAHA showed a similar effect to 3 weekly injections. These findings support that both single and 3 weekly IAHA are a practical treatment option for canine OA.

MANUFACTURERS

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***Ethical approval*.** This study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Kangwon National University and conducted after obtaining the owner's written consent.

***Declaration of interest*.** The authors declare no conflicts of interest related to this report. The authors alone are responsible for the content and writing of paper.

REFERENCES

1 **Alves J., Santos A.M.M.P., Jorge P., Lavrador C.F.T.V.B. & Carreira L.M. 2021.** Effect of a single intra-articular high molecular weight hyaluronan in a naturally occurring canine osteoarthritis model: a randomized controlled trial. *Journal of Orthopaedic Surgery and Research*. 16(1): 290.

2 **Aragon C.L., Hofmeister E.H. & Budsberg S.C. 2007.** Systematic review of clinical trials of treatments for osteoarthritis in dogs. *Journal of the American Veterinary Medical Association*. 230(4): 514-521.

3 **Bannuru R., Natov N., Dasi U., Schmid C. & McAlindon T. 2011.** Therapeutic trajectory following intra-articular hyaluronic acid injection in knee osteoarthritis–meta-analysis. *Osteoarthritis and Cartilage*. 19(6): 611-619.

4 **Black L.L., Gaynor J., Gahring D., Adams C., Aron D., Harman S., Gingerich D.A. & Harman R. 2007.** Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dogs with chronic osteoarthritis of the coxofemoral joints: a randomized, double-blinded, multicenter controlled trial. *Veterinary Therapeutics*. 8(4): 272-284.

5 **Bland S.D. 2015.** Canine osteoarthritis and treatments: a review. *Veterinary Science Development*. 5(2): 5931

6 **Brown D.C., Bell M. & Rhodes L. 2013.** Power of treatment success definitions when the Canine Brief Pain Inventory is used to evaluate carprofen treatment for the control of pain and inflammation in dogs with osteoarthritis. *American Journal of Veterinary Research*. 74(12): 1467-1473.

7 **Brown D.C., Boston R.C., Coyne J.C. & Farrar J.T. 2008.** Ability of the canine brief pain inventory to detect response to treatment in dogs with osteoarthritis. *Journal of the American Veterinary Medical Association*. 233(8): 1278-1283.

8 **Carapeba G.O., Cavaleti P., Nicácio G.M., Brinholi R.B., Giuffrida R. & Cassu R.N. 2016.** Intra-articular hyaluronic acid compared to traditional conservative treatment in dogs with osteoarthritis associated with hip dysplasia. *Evidence-Based Complementary and Alternative Medicine*. 2016: 2076921.

9 **Ghosh P. & Guidolin D. 2002.** Potential mechanism of action of intra-articular hyaluronan therapy in osteoarthritis: are the effects molecular weight dependent? *Seminars in Arthritis and Rheumatism*. 32(1): 10-37

10 **Hampshire V.A., Doddy F.M., Post L.O., Koogler T.L., Burgess T.M., Batten P.O., Hudson R., McAdams D.R. & Brown M.A. 2004.** Adverse drug event reports at the United States food and drug Administration Center for Veterinary Medicine. *Journal-American Veterinary Medical Association*. 225(4): 533-536.

11 **Henrotin Y., Sanchez C. & Balligand M. 2005.** Pharmaceutical and nutraceutical management of canine osteoarthritis: present and future perspectives. *The Veterinary Journal*. 170(1): 113-123.

12 **Johnston S.A., McLaughlin R.M. & Budsberg S.C. 2008.** Nonsurgical management of osteoarthritis in dogs. *Veterinary Clinics of North America: Small Animal Practice*. 38(6): 1449-1470.

13 **KuKanich B., Bidgood T. & Knesl O. 2012.** Clinical pharmacology of nonsteroidal anti-inflammatory drugs in dogs. *Veterinary Anaesthesia and Analgesia*. 39(1): 69-90.

14 **Kuroki K., Cook J.L. & Kreeger J.M. 2002.** Mechanisms of action and potential uses of hyaluronan in dogs with osteoarthritis. *Journal of the American Veterinary Medical Association*. 221(7): 944-950.

15 **Kuyinu E.L., Narayanan G., Nair L.S. & Laurencin C.T. 2016.** Animal models of osteoarthritis: classification, update, and measurement of outcomes. *Journal of Orthopaedic Surgery and Research*. 11(1): 19.

16 **Lee M.I., Kim J.H., Kwak H.H., Woo H.M., Han J.H., Yayon A., Jung Y.C., Cho J.M. & Kang B.J. 2019.** A placebo-controlled study comparing the efficacy of intra-articular injections of hyaluronic acid and a novel hyaluronic acid-platelet-rich plasma conjugate in a canine model of osteoarthritis. *Journal of Orthopaedic Surgery and Research*. 14(1): 314.

17 **Liu W., Burton-Wurster N., Glant T.T., Tashman S., Sumner D.R., Kamath R.V., Lust G., Kimura J.H. & Cs-Szabo G. 2003.** Spontaneous and experimental osteoarthritis in dog: similarities and differences in proteoglycan levels. *Journal of Orthopaedic Research*. 21(4): 730-737.

18 **Marshall K., Manolopoulos V., Mancer K., Staples J. & Damyanovich A. 2000.** Amelioration of disease severity by intraarticular hylan therapy in bilateral canine osteoarthritis. *Journal of Orthopaedic Research*. 18(3): 416-425.

19 **McArthur B.A., Dy C.J., Fabricant P.D. & Della Valle A.G. 2012.** Long term safety, efficacy, and patient acceptability of hyaluronic acid injection in patients with painful osteoarthritis of the knee. *Patient Preference and Adherence*. 6: 905-910.

20 **Nganvongpanit K., Boonsri B., Sripratak T. & Markmee P. 2013.** Effects of one-time and two-time intra-articular injection of hyaluronic acid sodium salt after joint surgery in dogs. *Journal of Veterinary Science*. 14(2): 215-222.

21 **Pashuck T.D., Kuroki K., Cook C.R., Stoker A.M. & Cook J.L. 2016.** Hyaluronic acid versus saline intra-articular injections for amelioration of chronic knee osteoarthritis: a canine model. *Journal of Orthopaedic Research*. 34(10): 1772-1779.

22 **Piirainen K. 2013.** Intra-articular injections in the management of canine osteoarthritis: a review of the literature. Available from:<http://urn.fi/URN:NBN:fi-fe201804208295>.

23 **Sandersoln R., Beata C., Flipo R.M., Genevois J.P., Macias C., Tacke S., Vezzoni A. & Innes J. 2009.** Systematic review of the management of canine osteoarthritis. *Veterinary Record*. 164(14): 418-424.

24 **Silva Jr. J.I., Rahal S.C., Santos I.F., Martins D.J., Michelon F., Mamprim M.J., Tomacheuski R.M. & Correia L.E. 2020.** Use of reticulated hyaluronic acid alone or associated with ozone gas in the treatment of osteoarthritis due to hip dysplasia in dogs. *Frontiers in Veterinary Science*. 7: 265.

25 **Suppan V.K.L., Tew M.M., Wong B.C., Chan H.K., Chew Y.W., Tan C.S., Nanta Kumar V.K., Shafie A.A. & Sadashiva Rao A. 2020.** One-year follow-up of efficacy and cost of repeated doses versus single larger dose of intra-articular hyaluronic acid for knee osteoarthritis. *Journal of Orthopaedic Surgery*. 28(1): 2309499019895029.

26 **Trairatthanom J., Trairatthanom S. & Thengchaisri N. 2019.** A retrospective study of the effect of single dose intra-articular hyaluronic acid injection on postoperative recovery in dogs undergoing stifle surgery. *The Thai Journal of Veterinary Medicine.* 49(4): 319-326.

27 **Wenz W., Breusch S., Graf J. & Stratmann U. 2000.** Ultrastructural findings after intraarticular application of hyaluronan in a canine model of arthropathy. *Journal of Orthopaedic Research*. 18(4): 604-612.

28 **Zóboli A.A.C., Rezende M.U., Campos G.C., Pasqualin T., Frucchi R. & Camargo O.P. 2013.** Prospective randomized clinical trial: single and weekly viscosupplementation. *Acta Ortopédica Brasileira*. 21(5): 271-275.