Hyaluronic Acid Versus Saline Intra-Articular Injections for Amelioration of Chronic Knee Osteoarthritis: A Canine Model

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ABSTRACT: The objective of this study was to assess the safety and efficacy of intra-articular injections of hyaluronic acid (HA) versus saline for symptomatic treatment of osteoarthritis (OA). Twenty-five adult purpose-bred dogs underwent meniscal release of one knee. Clinical, arthroscopic, and radiographic signs of OA were confirmed in all dogs prior to treatment. Dogs were randomized into five groups: HA-1 ($n = 5$), HA-3 ($n = 5$), HA-5 ($n = 5$), Saline-1 ($n = 5$), and Saline-3 ($n = 5$). Each dog received intra-articular injections of the respective substance into the affected knee at the pre-determined time points. Dogs were assessed for heat, swelling, and erythema after each injection and for lameness, pain, effusion, range of motion, kinetics, radiographic OA scoring, and arthroscopic scoring prior to treatment and for 6 months after injection. Dogs were then humanely euthanized and the knees assessed grossly and histologically. Only mild heat, swelling, and/or erythema were noted in some dogs following injection and resolved within 1 week. Dogs treated with HA-1, HA-3, and HA-5 were significantly ($p < 0.05$) better than dogs treated with Saline-1 and Saline-3 at the 4, 8, and 12 week time points based on at least one outcome measure. OA severity was not significantly different among groups at any time point, but increased in severity over time in all groups. Gross and histologic OA scores were not significantly different among groups. These data suggest the three HA injection protocols were safe, superior to saline for short-term amelioration of symptoms associated with chronic OA, and can be translated to human OA treatment. © 2016 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 34:1772–1779, 2016.

Keywords: osteoarthritis; knee; hyaluronic acid; animal model; canine

As the incidence of knee osteoarthritis (OA) continues to rise, safe therapies that limit disability are ever more important.[1–3] Treatment of knee OA with intra-articular hyaluronic acid (IAHA)—also referred to as viscosupplementation—remains a controversial topic in the current literature.[4–12] Clinical practice guidelines for IAHA are conflicting, ranging from recommendations for to recommendations against its use, with most remaining uncertain.[13–16] The safety of IAHA is generally accepted, however, efficacy remains highly debated. With respect to best current evidence for clinical efficacy, differences in study designs and HA formulations have made it difficult to make definitive conclusions. The 2013 AAOS clinical practice guidelines strongly recommend against using HA for patients with symptomatic knee OA.[15]

HA product characteristics differ in regards to origin, molecular weight, viscosity, residence times, and cross-linking. Treatment regimens range from single to multiple injections of varying frequency. Advancements with HA synthesis have yielded molecular weights that approach physiologic levels through cross-linking. High molecular weight (HMW) cross-linked HA has been shown to be more effective in improving the viscoelastic behavior of synovial fluid ex vivo.[17] More recent in vivo evidence has supported an inverse relationship between pain and HA molecular weight.[18–19]

The anatomy, histology, and biochemistry of the canine stifle (knee) closely resemble that of the human knee. Furthermore, causes, symptoms, and therapies for OA in dogs mimic those in people. As such, canine models for the study of OA are common and well accepted.[20–25] Meniscal release is one method of inducing OA as a pre-clinical model in dogs that consistently leads to lameness, effusion, loss in range of motion, and radiographic, arthroscopic and histologic evidence of OA by 12 weeks postoperatively. These similarities to human patients with knee OA make this canine model useful for translational research that requires controlled and comprehensive outcomes assessments.[20–25]

The objective of this study was to compare the safety and efficacy of intra-articular injections of HMW HA, low molecular weight (LMW) HA, and saline controls for symptomatic treatment of OA using the meniscal release model in dogs. This study compared five canine cohorts including a single Hylan G-F 20 injection, three weekly Hylan G-F 20 injections, five weekly sodium hyaluronate injections, a single saline injection, and three weekly saline injections. Functional methods of assessment included pain scores, effusion, comfortable range of motion (CROM), lameness, and kinetics. Radiographic, arthroscopic, histologic, and gross pathology were also assessed over the 6-month post-treatment study period. We hypothesized that all HA formulations would be safe and would provide superior clinical outcomes compared to saline controls.

MATERIALS AND METHODS

All procedures were approved by our institution's Animal Care and Use Committee. Twenty-five adult hound-mix

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(2–5 years of age, body weight mean = 27.4 kg, range = 21.2–31.6 kg; Marshall Farms BioResources, North Rose, NY, 145 USDA #21-A-008) purpose-bred research dogs were used. The dogs were allowed a 7-day acclimation period in the housing facilities prior to start of the study.

Preoperative Assessments

Orthopedic examination by a board-certified veterinary orthopaedic surgeon was performed on each dog prior to inclusion in the study. All limbs were assessed to ensure that no pre-existing orthopaedic disorders were evident. Knee comfortable range of motion (CROM) was measured using a standard goniometer, as previously described.[22–25] Clinical lameness scores were determined for each dog based on visual examination of gait by the same board-certified veterinary orthopaedic surgeon using a 10 cm visual analogue scale (VAS) and a validated grading system[22,24–27]:

0—no observable lameness

1—intermittent, mild weightbearing lameness with little, if any, change in gait

2—moderate weightbearing lameness—obvious lameness with noticeable gait change

3—severe weightbearing lameness—"toe-touching" only

4—non-weightbearing

Knee pain and knee effusion were assessed subjectively based on a VAS scale and recorded for each hindlimb of each dog, as previously described.[22–26]

Craniocaudal (anterioposterior) and mediolateral radiographic views of both knee joints of each dog were obtained, prior to synovial fluid collection and arthroscopic evaluation, and scored by one board-certified veterinary radiologist, using a modified subjective scoring system.[25] Nine regions within both knees were examined: femoral trochlea, medial femoral condyle, lateral femoral condyle, medial femoral epicondyle, lateral femoral epicondyle, proximal patella, distal patella, lateral tibial condyle, and medial tibial condyle. Each region was given a score of 0 (normal) to 3 (severe) for each knee joint. In addition, joint effusion/inflammation was scored using the same 0–3 categorical range. Scores for each knee were added to determine the total radiographic OA score for the joint. A total score of 1–10 indicates "Mild OA," a score of 11–20 indicates "Moderate OA," and a score of 21–30 indicates "Severe OA."[25]

Meniscal Release

On the day of surgery, dogs were pre-medicated, anesthetized, and prepared for aseptic surgery of the knee using a hanging limb technique. After draping, standard portals were created in the right knee for arthroscopic assessment, to confirm lack of pre-existing pathology, and meniscal release. A sterile meniscal knife was inserted through the instrument portal and used to create a complete radial transection of the medial meniscus at the caudal (posterior) horn junction with the caudal meniscotibial ligament.[22] Complete radial transection was confirmed by arthroscopic inspection (Fig. 1).

Postoperative recovery was directly monitored and analgesics (morphine 0.5 mg/kg IM) were administered to the dogs as needed for signs of pain for 48 h after surgery. The dogs were returned to their individual kennels and allowed unrestricted activity in the 18–25 square foot runs. In addition, the dogs were walked on a leash 5 days each week for 15 min at a pace to ensure use of all four limbs.

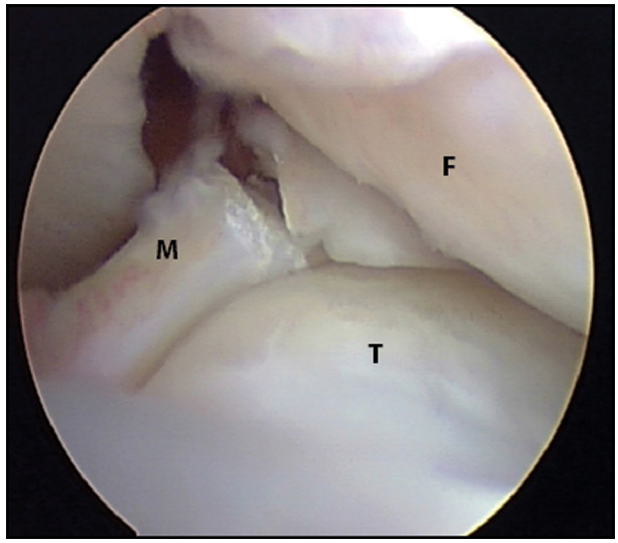

Figure 1. Arthroscopic medial meniscal release: complete radial transection of the meniscus in the caudal horn near its junction with the caudal meniscotibial ligament. T, tibia; M, meniscus; F, femur.

A physical examination was performed daily for the first 72 h after surgery and any observations recorded, including general condition, rectal temperature, appetite, and activity. The operated knee was observed daily for signs of swelling, erythema, and dehiscence until suture removal (7 days). Throughout the study the dogs were observed daily. The dogs were maintained in this way for 4 months prior to treatment in order to establish chronic OA in the MR knees.[22]

Pre-Treatment Assessments

Four months after MR (Pre), orthopedic examination to assess knee CROM, knee pain, knee effusion, clinical lameness, and function scoring were performed on each dog.[22] Kinetics assessment was performed using the GAITFour forcemat system (GAITFour, Haverton, Pennsylvania).[28–29] Craniocaudal and mediolateral radiographic views of the right knee of each dog were obtained, prior to synovial fluid collection and arthroscopic evaluation, and scored by one board-certified veterinary radiologist blinded to treatment, using a modified subjective scoring system.[22] Arthroscopic assessment of the right knee of each dog was performed. All articular surfaces of the patella, femur, and tibia were examined, scored, and mapped with respect to degree of articular cartilage damage using the International Cartilage Repair Society (ICRS) cartilage injury classification system.[30–32]

Intra-Articular Treatments

We developed a canine treatment protocol that paralleled the manufacturers' directions for human treatment and accounted for the volumetric size discrepancy between the canine stifle and the human knee. Treatment assignments were randomized so that investigators remained blinded to treatment throughout the study.

Under sedation with analgesia, the right knee of each dog underwent aseptic arthrocentesis using an 18 gauge needle

and 3 ml syringe to collect synovial fluid and ensure injections were intra-articular. The volume of the aspirated fluid was recorded. Each right knee was then aseptically injected intra-articularly through the same needle used for arthrocentesis as follows:

- Hyaluronic Acid (HA)-1 Group ($n = 5$): 3 ml of SYNVISC® 1 (Genzyme, Cambridge, Massachusetts) were injected once 4 months following MR;
- Hyaluronic Acid (HA)-3 Group ($n = 5$): 1 ml of SYNVISC® 1 (Genzyme, Cambridge, Massachusetts) was injected weekly for a total of three injections beginning 4 months following MR;
- Hyaluronic Acid (HA)-5 Group ($n = 5$): 1 ml of Hyalgan® 1 (Fidia, Parsippany, New Jersey) was injected weekly for a total of five injections beginning 4 months following MR;
- Control (Saline-1) Group ($n = 5$): 3 ml of sterile 0.9% saline were injected once 4 months following MR;
- Control (Saline-3) Group ($n = 5$): 1 ml of sterile 0.9% saline was injected weekly for a total of three injections beginning 4 months following MR.

The dogs were recovered and returned to their individual kennels and allowed to resume unrestricted activity in the 18–25 square foot runs and exercise (dogs were walked on a leash 5 days each week for 15 min at a pace to ensure use of all four limbs for the duration of the study).

Post-Treatment Assessments

Kinetics Assessment

At weeks 1, 4, 8, 12, 16, 20, and 24 after completion of treatment, kinetics assessment was recorded using a pressure-sensing walkway (GAITFour, Haverton, Pennsylvania), as previously described.[28–29] Dogs were trotted on the walkway until three complete data sets were obtained. Mean percent body weight distribution (%BW) was determined for each limb using the three complete data sets based on total pressure index (TPI). The %BW for the operated limb was chosen a priori as the variable for reporting. Dogs were walked across the portable walkway system in each direction with the handler attempting to maintain a consistent velocity on a loose leash. At least three acceptable passes (3–5 gait cycles), with video documentation, were obtained for each dog at each time point. Passes were included for analysis when the dogs walked at a steady pace with all four footfalls recorded for at least three gait cycles. The software program was used to distinguish the paw print for each footfall, which were then identified manually as left front, right front, left hind, or right hind accordingly.

Orthopedic Examination

Orthopedic examination to assess knee CROM, knee pain, knee effusion, clinical lameness, and function assessments were performed on each dog at weeks 1, 4, 8, 12, 16, 20, and 24 after completion of treatment. A single veterinarian, blinded to treatment, performed these analyses at all time points.

Radiographic Assessment

Under sedation with analgesia, radiographic assessments of the knee joints were performed on each dog at weeks 12 and 24 after completion of treatment. A single radiologist, blinded to treatment, analyzed all of the radiographic images at all time points.

Arthroscopic Examination

At week 24, after completion of treatment, arthroscopic assessment of the right knee of each was performed. All articular surfaces of the patella, femur, and tibia were examined, scored, and mapped with respect to degree of articular cartilage damage using the ICRS cartilage injury classification system. Meniscal pathology was also arthroscopically assessed and described in terms of nature, extent, and location.

Post-Mortem Assessments

At 24 weeks, after completion of treatment, dogs were humanely euthanatized. Full necropsy was performed immediately after euthanasia by a board-certified veterinary pathologist, who was blinded to treatment group and clinical findings. Both knees from each dog were carefully dissected to assess gross pathology of the articular cartilage, cruciate ligaments, and menisci. Macroscopic alterations of synovium (e.g., thickening/fibrosis, discoloration, and vascularity) were scored using the scoring system set forth in the OARSI histopathology initiative.[31] Portions of the synovium were excised and placed in formalin in preparation for histologic processing.

Histologic Assessments

The proximal end of the operated tibia and the distal end of the operated femur were removed and placed into 10% neutral buffered formalin. Bones were allowed to fix for 5 days and then placed in an Immunocal™ decalcifier (StatLab Medical Products, McKinney, Texas). After decalcification was complete, the medial and lateral femoral condyles and medial and lateral tibial condyles were each divided into three sections approximately 2–4 mm thick for processing, embedding in paraffin, microtome sectioning (8 μm) and staining (H&E and Toluidine Blue). Histologic scoring of the osteochondral tissues was performed by one board-certified veterinary pathologist, blinded to treatment, using the OARSI histologic scoring system for canine osteoarthritis. Synovial tissue was routinely processed, sectioned (5 μm) and stained (H&E), and scored using the OARSI histologic scoring system for canine OA.[31]

Statistical Analyses

For CROM measurements, the difference between right and left limbs was calculated and used for statistical analyses. Mean ± standard deviation (SD) was determined for each outcome measure, time point, and group. Within group comparisons over time were done using repeated measures ANOVA for continuous data or repeated measures ANOVA on ranks for categorical data. Among group comparisons were done using one-way ANOVA for continuous data or ANOVA on ranks for categorical data. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

All 25 dogs successfully underwent meniscal release, were assigned injection treatment, and survived for the intended duration of the study.

Meniscal Release Model of OA

No evidence for lameness or OA was present in any dog prior to MR. However, MR successfully induced clinical signs of lameness and OA by the time of intra-articular treatment (Table 1). Arthroscopic assessment

Table 1. Mean ± SD Values for Outcome Measures Assessed Prior to and After Meniscal Release in This Study

	Lameness	Function	CROM	Pain	Effusion	XR OA
Pre-MR	0 ± 0	10 ± 0	104.5° ± 2.6	0 ± 0	0 ± 0	0 ± 0
4 months after MR	2.0 ± 0.0	5.9 ± 0.5	82.6° ± 6.5	3.2 ± 0.7	3.2 ± 0.9	8.8 ± 3.3

No evidence for lameness or OA was present in any dog prior to MR. However, MR successfully induced clinical signs of lameness and OA by the time of intra-articular treatment. There were no significant differences among groups with respect to measures of kinetics, lameness, function, knee CROM, pain, effusion, or radiographic assessments at the time of treatment.

of the operated knees performed prior to treatment showed consistent meniscal subluxation, medial femoral, and medial tibial articular cartilage pathology (grade 2–4, on the 5-point ICRS scale), and mild to moderate synovitis. There were no significant differences among groups with respect to measures of kinetics, lameness, function, knee CROM, pain, effusion, radiographic, or arthroscopic assessments at the time of treatment.

Adverse Events After Treatment

Only mild changes in heat, swelling, and/or erythema were noted in some dogs following injection (HA-1 [$n = 2$], HA-3 [$n = 1$], HA-5 [$n = 2$], Saline-1 [$n = 1$], Saline-3 [$n = 2$]) and all resolved within 1 week without need for additional treatment.

Outcomes Post-Treatment/Injection

Lameness, Function, and Kinetics

HA-1 and HA-3 dogs were significantly ($p < 0.05$) less lame with higher function and TPI than saline controls at all time points after completion of treatment. HA-1 and HA-3 dogs were significantly ($p < 0.05$) less lame with higher function and TPI than HA-5 dogs at 4 and 20 weeks after completion of treatment. HA-5 dogs were significantly ($p < 0.05$) less lame with higher function and TPI than saline controls at 4, 8, 12, and 16 weeks after completion of treatment. HA-1 and HA-3 dogs were not significantly ($p > 0.2$) different from one another with respect to lameness grade, level of function, or TPI at any time point. HA-1 and HA-3 dogs were significantly ($p < 0.05$) less lame with higher function and TPI compared to their pre-treatment values at all time points post-treatment except 1 and 24 weeks after completion of treatment. HA-5 dogs were significantly ($p < 0.05$) less lame with higher function and TPI compared to their pre-treatment values at 4, 8, 12, and 16 weeks after completion of treatment. Saline-1 and Saline-3 dogs were not significantly ($p > 0.4$) different from one another or their pre-treatment values with respect to lameness grade, level of function, or TPI at any time point. Lameness grade, level of function, and TPI corresponded well to each other and produced the same results with respect to statistical significance, therefore, TPI data are provided in Figure 2 since they are objective and quantitative.

Pain, Effusion, and Range of Motion

HA-1, HA-3, and HA-5 dogs were significantly ($p < 0.05$) less painful than saline controls at all time points post-treatment except 24 weeks after completion of treatment. HA-1, HA-3, and HA-5 dogs were not significantly ($p > 0.1$) different from one another with respect to level of pain at any time point. HA-1, HA-3, and HA-5 dogs were significantly ($p < 0.05$) less painful compared to their pre-treatment values at all time points post-treatment except 24 weeks after completion of treatment. Saline-1 and Saline-3 dogs were not significantly ($p > 0.4$) different from one another or their pre-treatment values with respect to level of pain at any time point (Fig. 3).

No statistically significant ($p > 0.3$) differences in level of effusion were noted within any group over time or among groups at any time point.

HA-1, HA-3, and HA-5 dogs had significantly ($p < 0.05$) less CROM difference (better range of motion in the affected knee) than saline controls at 4, 8, 12, and 16 weeks after completion of treatment. HA-1, HA-3, and HA-5 dogs were not significantly ($p > 0.2$) different from one another with respect to CROM difference at any time point. HA-1, HA-3, and HA-5 dogs had significantly ($p < 0.05$) less CROM difference compared to their pre-treatment values at 1, 4, 8, 12, and 16 weeks after completion of treatment. Saline-1 and Saline-3 dogs were not significantly ($p > 0.4$) different from one another or their pre-treatment values with respect to CROM difference at any time point (Fig. 4).

Radiographic OA

Radiographic OA severity increased in all groups over the 24-week evaluation period after completion of treatment from the high "mild" to low "moderate" range. However, the differences in severity were not statistically significant ($p > 0.2$) within any group over time or among groups at any time point (Fig. 5).

Arthroscopic Assessment

Based on arthroscopic assessment of MR joints performed prior to treatment and at 24 weeks after completion of treatment, all groups had significantly ($p < 0.01$) more articular cartilage pathology at week 24 compared to the pre-treatment time point. Among groups, no statistically significant ($p > 0.6$) differences were noted at either time point. For all groups and all time points, cartilage pathology was most severe in the medial compartment (medial femoral and tibial condyles) (Fig. 6).

Gross Assessment

Based on gross assessment of the treated knees at 24 weeks after completion of treatment, all groups had

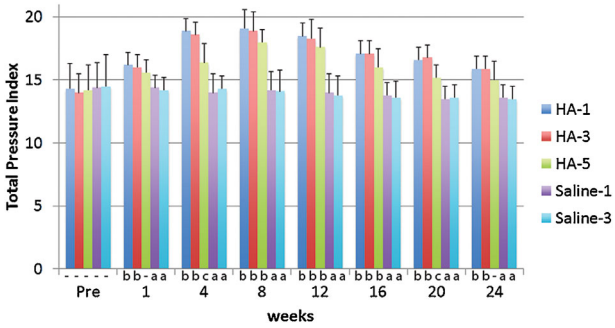

Figure 2. Mean ± SD values for %Total Pressure Index of the affected hindlimb for dogs in the HA and saline treatment groups over the 24-week study period. HA-1 and HA-3 dogs had significantly ($p < 0.05$) higher %TPI compared to saline controls at all time points post-treatment. HA-1 and HA-3 dogs had significantly higher ($p < 0.05$) %TPI than HA-5 dogs at 4 and 20 weeks post-treatment. HA-5 dogs had significantly ($p < 0.05$) higher %TPI than saline controls at 4, 8, 12, and 16 weeks post-treatment. Saline-1 and Saline-3 dogs were not significantly ($p > 0.4$) different from one another or their pre-treatment values with respect to %TPI at any time point. HA, hyaluronic acid. [a–b] Comparisons involving different letters (e.g., a/b) indicate significant ($p < 0.05$) differences among groups at each time point. Comparisons involving similar letters (e.g., a/a) and no letters (e.g., –/b, –/–) indicate nonsignificance.

moderate to severe articular cartilage damage (predominantly in the medial compartment), mild to moderate synovitis, and medial meniscal subluxation and fibrillation.

Histologic Assessment

Histologic scoring of synovial pathology revealed no statistically significant differences ($p = 0.83$) among groups. Histologic scoring of osteochondral pathology revealed no statistically significant differences ($p = 0.91$) among groups (Fig. 7).

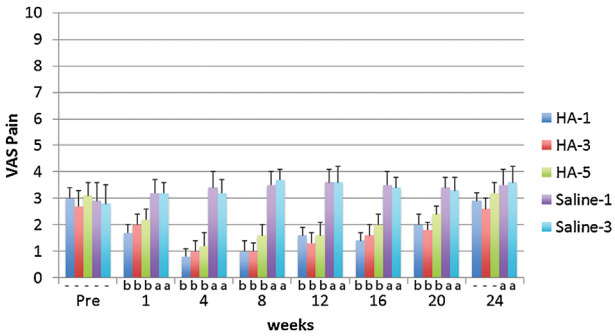

Figure 3. Mean ± SD values for VAS pain in the affected knees of dogs in the HA and saline treatment groups over the 24-week study period. HA-1, HA-3, and HA-5 dogs were significantly ($p < 0.05$) less painful than saline controls and pre-treatment values at all time points post-treatment except 24 weeks. HA-1, HA-3, and HA-5 dogs were not significantly ($p > 0.1$) different from one another with respect to level of pain at any time point. Saline-1 and Saline-3 dogs were not significantly ($p > 0.4$) different from one another or their pre-treatment values with respect to level of pain at any time point VAS, visual analog scale; HA, hyaluronic acid. [a–b] Comparisons involving different letters (e.g., a/b) indicate significant ($p < 0.05$) differences among groups at each time point. Comparisons involving similar letters (e.g., a/a) and no letters (e.g., –/b, –/–) indicate nonsignificance.

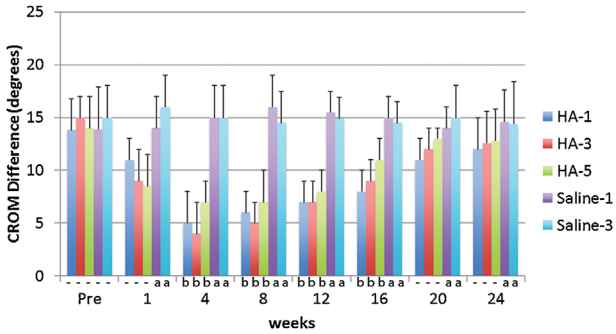

Figure 4. Mean ± SD values for comfortable range of motion (CROM) in the affected knees of dogs in the HA and saline treatment groups over the 24-week study period. HA-1, HA-3, and HA-5 dogs had significantly ($p < 0.05$) less CROM difference (better range of motion in the affected knee) than saline controls at 4, 8, 12, and 16 weeks post-treatment. HA-1, HA-3, and HA-5 dogs were not significantly ($p > 0.2$) different from one another with respect to CROM difference at any time point. HA-1, HA-3, and HA-5 dogs had significantly ($p < 0.05$) less CROM difference compared to their pre-treatment values at 1, 4, 8, 12, and 16 weeks post-treatment. Saline-1 and Saline-3 dogs were not significantly ($p > 0.4$) different from one another or their pre-treatment values with respect to CROM difference at any time point. HA, hyaluronic acid. [a–b] Comparisons involving different letters (e.g., a/b) indicate significant ($p < 0.05$) differences among groups at each time point. Comparisons involving similar letters (e.g., a/a) and no letters (e.g., –/b, –/–) indicate nonsignificance.

DISCUSSION

IAHA injections in dogs with established knee OA resulted in clinically significant improvements with respect to pain, function, lameness, kinetics, and CROM when compared to pre-treatment values and saline controls for 4–6 months after treatment. Single or series of three injections of higher molecular weight HA were associated with the most notable and sustained beneficial effects. The efficacy and duration of IAHA in this study were consistent with previous reports assessing IAHA in human knee OA.[5,8,10,12,33] Maximum benefit was noted at 4–8 weeks after injection and gradually tapered back toward pre-treatment values by the 6-month time point. Given the similarities of the canine MR model to human knee OA, these findings support the safety and efficacy of

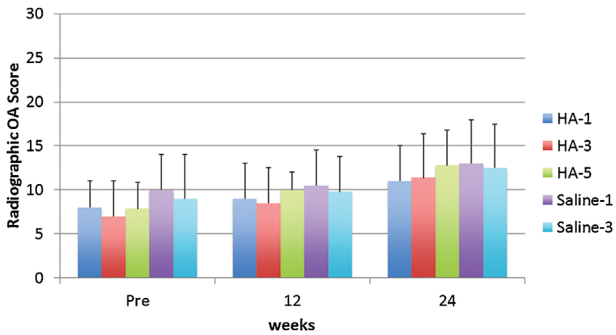

Figure 5. Mean ± SD values for radiographic OA severity scores in the affected knees of dogs in the HA and saline treatment groups. Radiographic OA severity increased in all groups over the 24-week evaluation period post-injection from the high "mild" to low "moderate" range. However, the differences in severity were not statistically significant ($p > 0.2$) within any group over time or among groups at any time point. OA, osteoarthritis; HA, hyaluronic acid.

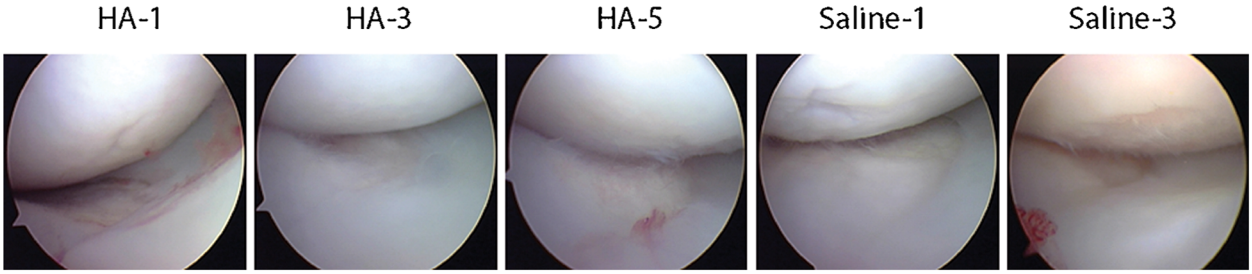

Figure 6. Representative arthroscopic images obtained 24-weeks after treatment. Based on arthroscopic assessment of MR joints performed prior to treatment and at 24 weeks post-treatment, all groups had significantly ($p < 0.01$) more articular cartilage pathology at week 24 compared to the pre-treatment time point. Among groups, no statistically significant ($p > 0.6$) differences were noted at either time point. For all groups and all time points, cartilage pathology was most severe in the medial compartment (medial femoral and tibial condyles). MR, meniscal release; HA, hyaluronic acid.

IAHA as a symptomatic treatment option for patients with knee OA.

A major question concerning clinical use of IAHA centers on whether HMW or LMW preparations are superior. Hyalgan® 1, the LMW HA used in this study, has a molecular weight of 500–730 kDA. SYNVISC® 1, the cross-linked HMW HA used in this study, has a MW of 6,000 kDA. The MW of HA in normal human synovial fluid ranges from 5,000–10,900 kDa.[34–35] In OA, synovial HA is depolymerized to 2,700–4,500 kDa and cleared from the joint more rapidly.[34–36] Clinically, this lower MW distribution is associated with an increased risk for rapid OA progression.[18] The present study demonstrated statistically significant advantages in functional limb use for the HMW groups compared to the LMW group, which occurred earlier and lasted longer. However, there were not statistically significant differences between preparations with respect to knee pain, range of motion, or any other outcome measures assessed with all HA groups being superior to saline controls.

Based on radiographic, arthroscopic, gross, and histologic assessments, none of the IAHA protocols were effective in ameliorating the development or progression of OA associated with MR in dogs. These findings are consistent with Smith et al., who used IAHA in a transection (ACLT) knee OA model in dogs. However, Marshall et al. reported that three weekly injections of Hylan G-F 20 starting two months after ACLT in dogs significantly decreased the severity of knee OA using gross and histological indices. Further, studies in rabbits suggest a chondroprotective effect when IAHA is administered shortly after insult.[37–38] Elmorsy et al. reported less OA progression, better friction coefficients, and improved histological scores, most notably affecting the superficial cartilage layer, when comparing HMW HA to saline controls administered 5 weeks after ACLT in rabbits. Kichuchi et al. reported similar findings with HMW HA being more effective than LMW HA in inhibiting cartilage degeneration when HA was administered immediately after meniscectomy in rabbits. The findings from the present study, which revealed no significant differences among groups for synovial or osteochondral pathology may be related to more chronic and symptomatic joint pathology at the time of therapeutic intervention, the longer study duration post-treatment (24 weeks vs. 2–4 weeks), differences among the animal models, and/or the nature of the outcome measures employed. Based on the current indications for use of IAHA in human patients, the present study mimics the clinical scenario most closely.

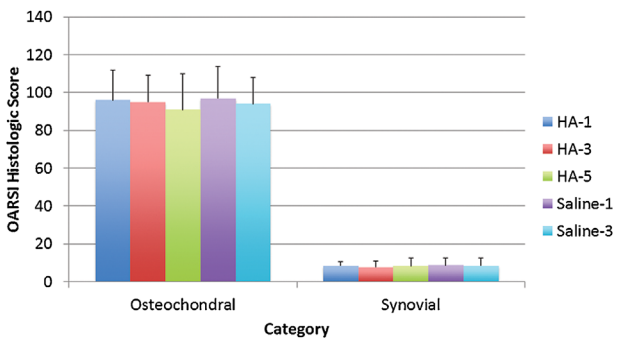

Figure 7. Mean ± SD values for whole-joint OARSI histologic assessments performed in this study. Histologic scoring of synovial pathology revealed no statistically significant differences ($p = 0.83$) among groups. Histologic scoring of osteochondral pathology revealed no statistically significant differences ($p = 0.91$) among groups. OARSI, osteoarthritis research society international; HA, hyaluronic acid.

Study limitations should be considered when translating these data for clinical applicability. The experimental design was based on a translational animal model, which is valid for pre-clinical study of OA therapeutics but does not exactly mimic the human situation. This may be most important with respect to placebo effect. The placebo effect associated with saline injections in human patients has been reported to be effective in the treatment of OA, especially for pain, stiffness, and self-reported function.[7,39] In the present animal model study, no significant placebo effects were noted. In addition, this study did not include additional treatment cohorts such as oral non-steroidal anti-inflammatory medications or intra-articular injections other than HA as it was designed to effectively assess current IAHA protocols in a placebo-controlled animal model. Importantly, safety testing was valid in this animal model in that

commercially available products were delivered using current clinical protocols. Only mild, self-limiting episodes of heat, swelling, and/or erythema were noted and were distributed among treatment and placebo groups. No serious adverse events were noted in this study. These findings match those noted for human patients and IAHA is generally considered safe for treatment of knee OA.[11–12,40–42]

CONCLUSIONS

These data suggest that currently used HA injection protocols were safe and were superior to saline for short-term amelioration of symptoms associated with chronic OA. IAHA injections resulted in clinically significant improvements with respect to pain, function, and range of motion for 4–6 months after treatment with high molecular weight HA showing the most notable and sustained beneficial effects. These findings support the safety and efficacy of IAHA as a symptomatic treatment option for patients with knee OA.

AUTHORS' CONTRIBUTIONS

All authors have read and approved the final submitted manuscript. The following is the author contribution: KK, CRC, AMS, JLC: substantial contributions to research design, acquisition, analysis of interpretation of data; TDP, KK, CRC, AMS, JLC: drafting the paper and revising it critically; TDP, KK, CRC, AMS, JLC: approval of the submitted and final versions.

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