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Intra-Articular Injection of High-Dose ELHLD Peptide for Managing Canine Stifle Osteoarthritis: Kinetic Gait Analysis

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Abstract Intra-articular injection of ELHLD peptide is considered to have a therapeutic effect in osteoarthritis (OA) through the inhibition of transforming growth factor–β1. This study aimed to assess the efficacy of intra-articular injections of high-dose ELHLD peptide (100 μg/kg) in canine stifle OA. Six client-owned dogs diagnosed with stifle OA were included. Selected dogs were treated with an intra-articular injection of high-dose ELHLD peptide (100 μg/kg). Outcome measures, including orthopedic examination, gait analysis, and Canine Brief Pain Inventory (CBPI) score, were evaluated four times after injection. Orthopedic examination, gait analysis, and owner's assessment (CBPI) improved significantly from 4 weeks after injection. In conclusion, we obtained sufficient evidence from this small sample that high-dose ELHLD peptide improves clinical signs of canine OA not only through subjective assessment but also through objective evaluation.

Key words dog, ELHLD peptide, osteoarthritis, kinetic gait analysis, stifle joint.

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Introduction

Osteoarthritis (OA) is one of the most common conditions in dogs that causes chronic pain and daily discomfort (13). OA is a progressive and degenerative disease in which several molecular signaling and kinase cascades are considered to play role in the complex etiology (19). Because the complete treatment of OA has not been established, the aims of therapy are to relieve pain and improve quality of life (11,22). The most commonly used pain relief treatment is administration of nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs contribute to reducing inflammation through the inhibition of cyclooxygenase (COX). COX not only can be the cause of the inflammation but also has systemic functions such as protecting gastric mucosa and regulating kidney blood flow. Therefore, the long-term use of COX-inhibiting agents (NSAIDs) can lead to gastrointestinal disorders and nephrotoxicity, which are considered major complications of NSAIDs (20). Because most patients diagnosed with OA are older than middle age, complications can be more serious. Therefore, recent studies have evaluated a number of intra-articular injection materials for OA (5-7,16).

In this study, we administered an intra-articular injection of ELHLD peptide, which binds with transforming growth factor-β1 (TGF-β1) in OA patients to selectively block the Smad 1/5/8 pathway (15). Among the TGF superfamily, TGF-β1 is known as promoting chondrogenesis and the production of cartilage extracellular matrix, which is important to maintain the homeostasis of articular cartilage (28,29,34,36). In a normal joint, TGF-β1 inhibits chondrocytic changes by acting on the Smad 2/3 pathway (12). In the OA joint, on the other hand, TGF-β1 is overexpressed and activates the Smad 1/5/8 pathway instead of the Smad 2/3 pathway. This alteration in pathway causes hypertrophy and transformation of chondrocytes into macrophage-like cells or apoptotic cells (29). This chondrocytic change leads to osteophyte formation and subchondral bone changes, which cause discomfort during movement and lead to a decrease in joint motion.

Previous studies have suggested that inhibition of TGF-β1 may have chondroprotective and regenerative effects in OA of human and rodents (24,26,35). Considering this potential efficacy, intra-articular injection materials that inhibit TGF-β1 have been used in canine OA, but the clinical evidence is still insufficient. Therefore, identifying the efficacy of high-dose ELHLD peptide (100 μg/kg) is the purpose in this study. We chose this dose because ELHLD peptide can be injected at doses as high as 3,000 μg per joint in dogs (15). In this study, we evaluated subjective and objective indices, including orthopedic examination, owner's assessment, and gait analysis.

Materials and Methods

Dog selection

All study procedures were reviewed and approved by Seoul National University Institutional Animal Care and Use Committee (SNU-200423-2). Six client-owned dogs diagnosed with stifle OA at Seoul National University Animal Hospital participated in the study. We included dogs exhibiting clinical signs of unilateral stifle OA with radiographic changes. Dogs with bilateral stifle OA but apparently having unilateral lameness were not excluded. We excluded dogs that underwent orthopedic surgery within 1 month and dogs treated with NSAIDs for at least 1 week, short-acting steroids for at least 2 weeks, and long-acting steroids for at least 6 months (17). The use of certain medications, including opioids, local medication considered to have an effect on OA, other intra-articular injection material, immune-suppressive medicine, corticosteroids, and anticoagulation, was prohibited during the entire study.

ELHLD peptide

We used JointVex® (Vexpert, Korea) for intra-articular injection, including ELHLD peptide 0.5 mg/vial, which consisted of a white powder formulation mixed appropriately with normal saline (0.9% NaCl). Depending on the patient's body weight, a concentration of 100 μg/kg, with a total amount of 0.1 to 0.5 mL, was injected into the joint.

Study protocol

Owners were provided with information on the procedures and ELHLD peptide. They signed a consent paper to participate in the study. Before treatment, we performed a general physical examination (weight, blood pressure, temperature, heart rate, respiration rate, body condition score) and blood analysis to exclude dogs with other systemic problems. Selected dogs according to inclusion or exclusion criteria underwent an orthopedic examination and gait analysis, and their owners performed the Canine Brief Pain Inventory (CBPI). We then administered an intra-articular injection of the stifle joint. All participants visited the hospital for reevaluation four times after the intra-articular injection. Fig. 1 summarizes the details of the procedures according to visit date.

Orthopedic examination

Each dog was assessed by a single trained veterinarian. Standing posture, lameness during walk and trot, weight bearing, joint mobility, pain on palpation, and clinical condition were scored preinjection and at 2, 4, 8, and 12 weeks after injection using a previously published modified scoring

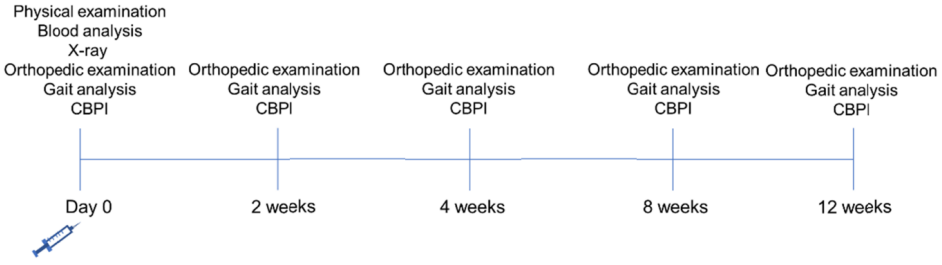

Fig. 1. Summary of the study protocol.

Table 1. Orthopedic examination criteria

Score criteria
1. Standing stance
[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 the floor)
[4] Severely abnormal stance (no weight-bearing)
2. 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
3. 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
4. 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
5. 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%)
6. Pain at palpation/mobilization
[1] No pain elicited on palpation/mobilization 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., vocalizes or becomes aggressive
[5] Severe pain elicited, e.g., not allow examiner to palpate/mobilize the joint
7. Evaluation of overall clinical condition
[1] Good
[2] Mildly poor
[3] Moderately poor
[4] Severely poor
[5] Very severely poor

ROM, range of motion.

criteria (18) (Table 1). In addition, the range of motion of the stifle joints using a goniometer and muscle mass of the hindlimb were measured at 1/4 of the proximal femur and 1/4 of the proximal tibia.

Owner's assessment

At each examination visit, the owners performed the CBPI score (32). The CBPI is a questionnaire, most frequently used in veterinary OA studies (1), composed of two categories of Pain Severity Score (PSS) and Pain Interference Score (PIS), consisting of 22 questions. Each question is rated on an 11-point numerical scale (0-10), and the overall result is calculated by summing the scores.

Gait analysis

We collected data using a low-profile pressure-sensitive walkway (Strideway™; Tekscan, South Boston, MA, United States) in a private room. Before the analysis, each dog was allowed to become familiar with the environment. They were then guided to walk straight over the walkway several times. We obtained data of the first five valid trials in which the dog did not have any obvious head turning or pulling on the leash. Recorded data were processed simultaneously using specific software (Strideway Research, Tekscan), providing normalized peak vertical force (PVF) and vertical impulse (VI) according to body weight. Symmetry index (SI) and weight distribution (WD) were calculated according to a previous published study show in the following formulas (4):

Symmetry index of PVF
$= (0.5 \times [PVFa - PVFc])/(PVFa - PVFc) \times 100\%$,
Symmetry index of VI
$= (0.5 \times [VIa - VIc])/(VIa - VIc) \times 100\%$,
Weight distribution of PVF
$= (PVFa/\text{total PVF of the four limbs}) \times 100\%$,
Weight distribution of VI
$= (VIa/\text{total VI of the four limbs}) \times 100\%$,

where PVFa is the mean PVF of the affected hindlimb, PVFc is the mean PVF of the contralateral hindlimb, VIa is the mean VI of the affected hindlimb, and VIc is the mean VI of the contralateral hindlimb.

Statistical analysis

We performed the statistical analysis using GraphPad Prism 8.01 for Windows (GraphPad Software, La Jolla, CA, USA). We compared the preinjection and postinjection OA scores using the Wilcoxon test. Statistical differences in orthopedic examination, CBPI score, and gait analysis between preinjection and each visit (2, 4, 8, and 12 weeks) were analyzed using the Friedman test with Dunn's post hoc analysis. For all statistical results, $p < 0.05$ was considered significant.

Results

General information

Six client-owned dogs were included (mean age 10.5 ± 2.5 years, mean body weight 5.5 ± 1.8 kg). All dogs were small breeds, including Yorkshire terrier (n = 3), Poodle (n = 2), and Maltese (n = 1), and were of both sexes (2 males, 4 females). Table 2 presents patient details. To exclude other general disorders before enrollment, we conducted a general physical examination and blood analysis for all individuals. No dogs demonstrated any remarkable findings on the physical or blood analyses. Subsequently, 100 μg/kg of ELHLD was injected, and all osteoarthritic lesions were on the right stifle joints. After all tests were complete, none of the patients had signs of side effects from the injection.

Orthopedic examination

We measured the muscle circumference of the hindlimb at each visit and compared the mean values of the change before and after injection. The timing of the muscle mass increase varied, but five dogs had an increase in muscle circumference. The mean circumference was increased after injection with 0.42 ± 0.2 cm at the proximal femur and 0.58 ± 0.38 cm at the proximal tibia (Table 3). The range of motion of the stifle joints was compared with the ratio of change before and after injection. The range of motion of the joints also increased by an average of 1.12 ± 0.05 times after injection (Table 4).

In analyzing the orthopedic examination score as evaluated by veterinarians, the sum indexed scores significantly improved in terms of clinical signs from 4 weeks postinjection in all dogs, five of which improved at 2 weeks postinjection (Fig. 2). In particular, in one case, all scores were evaluated as normal at 8 weeks postinjection and were maintained until

Table 2. Study population

Id	Age (years)	Breed	Weight (kg)	Gender	Affected joint
Dog 1	11	Yorkshire terrier	4.1	FS	Right stifle
Dog 2	15	Yorkshire terrier	8.5	MC	Right stifle
Dog 3	9	Poodle	4.7	FS	Right stifle
Dog 4	8	Yorkshire terrier	3.7	FS	Right stifle
Dog 5	11	Poodle	7.1	FS	Right stifle
Dog 6	9	Maltese	5.1	MC	Right stifle

FS, female spayed; MC, male castrated.

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the end of the study. For each index comparison, significant improvements were identified in the 2 and 4 index, indicating that lameness and weight bearing were improved after injection (Fig. 2).

Owner's assessment (CBPI)

The sum indexed scores of the CBPI indicated that all dogs significantly improved after the injection, with significant differences observed from 4 weeks postinjection (Fig. 3). In the

Table 3. Muscle circumference of hindlimb

Id	Pre-PF (cm)	Post-PF (cm)	Pre-PT (cm)	Post-PT (cm)	Change of PF (cm)	Change of PT (cm)
Dog 1	17.5	18	9	10	0.5	1
Dog 2	22	22.5	12	12	0.5	0
Dog 3	19	19.5	11.5	12	0.5	0.5
Dog 4	17	17	10	11	0	1
Dog 5	21	21.5	10	10.5	0.5	0.5
Dog 6	20	20.5	11.5	12	0.5	0.5

PF, Proximal femur; PT, proximal tibia.

Table 4. Motion of stifle joint at pre-injection and post-injection

Id	Pre-ROM (°)	Post-ROM (°)	Change ratio
Dog 1	120	130	1.08
Dog 2	105	125	1.19
Dog 3	125	140	1.12
Dog 4	125	130	1.08
Dog 5	120	130	1.08
Dog 6	115	130	1.17

ROM, Range of motion.

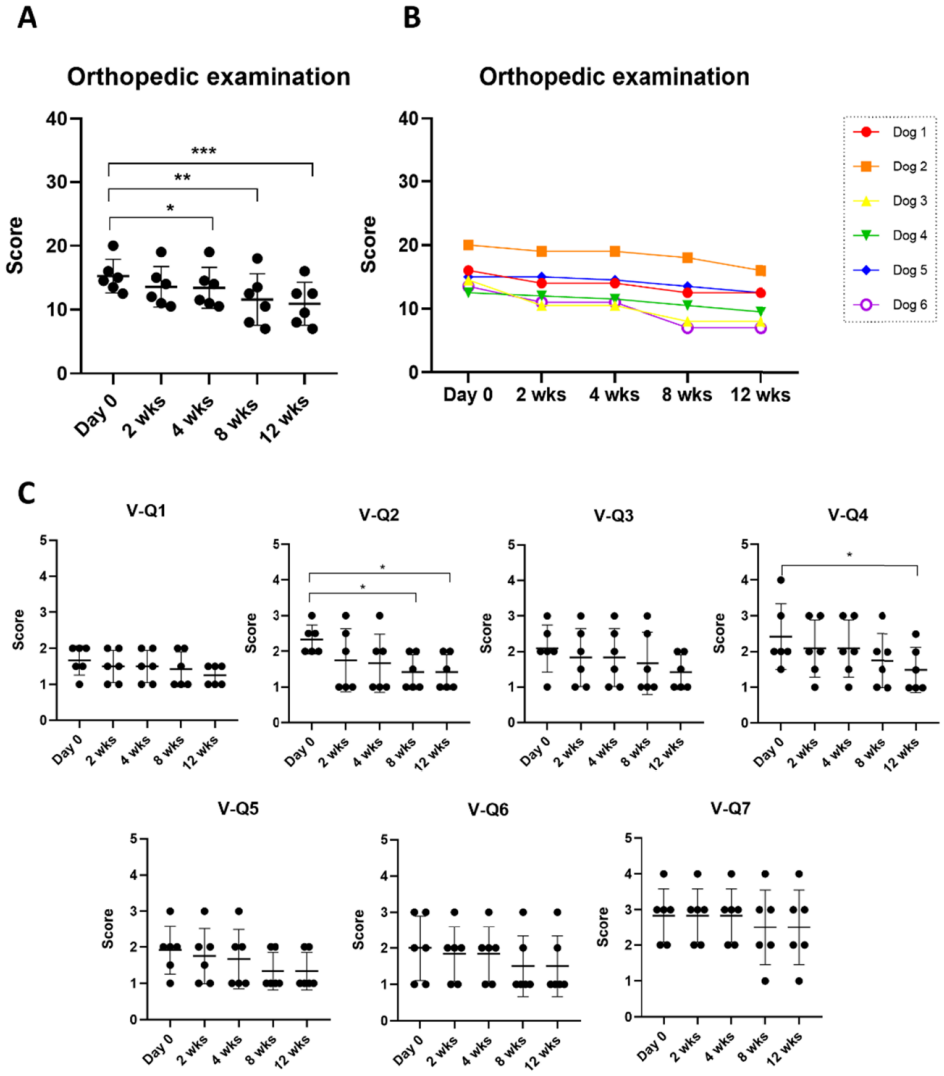

Fig. 2. Assessment of the orthopedic examination scores. Data are presented as median and range. (A) Orthopedic examination score of all dogs. (B) Individual trend of orthopedic examination score. (C) Each index of orthopedic examination score. *p < 0.05, **p < 0.01, ***p < 0.001 between each time point and day 0.

sum indexed PSS scores, significantly lower PSS scores were observed from week 4 after intra-articular injection, with a PSS of 11 at week 12, which indicated complete alleviation of OA pain, as evaluated by the dog's owner. Moreover, the sum indexed PIS scores indicated significantly lower PIS scores from 4 weeks postinjection; among them, three dogs were found to have improved their daily movement, as their scores at 12 weeks were less than half of their scores before injection (Fig. 3).

Gait analysis

The SI of PVF and VI was significantly improved at 4 weeks postinjection, and the WD of PVF and VI was significantly improved at 8 weeks postinjection (Fig. 4). In the individual analysis, five dogs had a tendency toward improvement of the SI of PVF and VI from 2 weeks postinjection and continued to improve until 12 weeks, of which three dogs presented an SI below 6, which is considered to be normal in dogs. In the individual WD analysis, there was a tendency toward improvement until 12 weeks (Fig. 4).

Discussion

In this study, high-dose ELHLD peptide was injected in six dogs diagnosed with stifle OA. This study provides preliminary evidence for the use of intra-articular injection of ELHLD peptide, in that significant improvement was seen in the subjective assessment (orthopedic examination score and CBPI) and in the objective analysis of weight bearing (SI of PVF and VI and WD of PVF and VI) in dogs with stifle OA.

In osteoarthritic joints, TGF-β1 is overexpressed and loses its normal functions. Several studies have reported that the overexpression of TGF-β1 is associated with cartilage degeneration (9,23,27,30,33), leading to osteophyte formation, hypertrophy of articular chondrocytes, and synovial fibrosis. Therefore, we used ELHLD peptide, which selectively inhibits TGF-β1, in dogs with OA in this study. Our results revealed an overall improvement after injection of high-dose ELHLD peptide for the management of canine OA, evaluated using both subjective and objective parameters. In addition, high-dose ELHLD peptide appears to be safe with no obvious adverse events observed in this study.

Evaluating the clinical signs of OA patients in veterinary medicine has been rudimentary and subjective (31). In this study, we used a modified orthopedic scoring system that includes more subdivided evaluation parameters for monitoring mild clinical symptoms and subtle changes. All orthopedic examination scores were significantly improved from 4 weeks after injection. We found no statistically significant improvement in the 2 weeks after injection, but five of the six dogs showed a decreased score. One dog that did not improve, however, had an falling accident a few days before the examination. According to the owner, the dog had walked well after the intra-articular injection before the accident. Fortunately, this dog recovered soon and was not excluded from this study. Among those examination scores, lameness at walk and weight bearing especially showed a significant difference after the injection. In addition, not only muscle

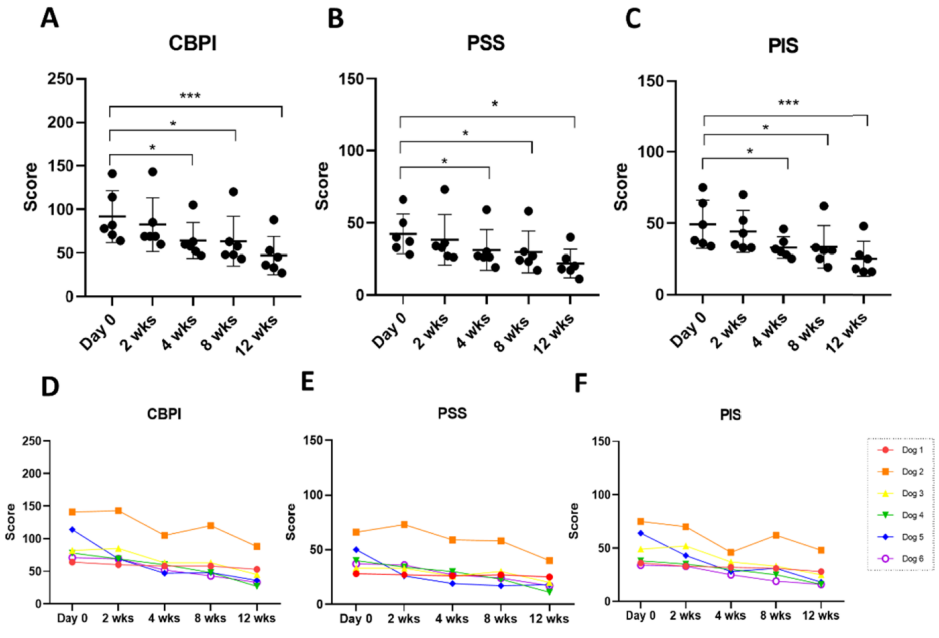

Fig. 3. Assessment of the Canine Brief Pain Inventory (CBPI) score. Data are presented as median and range. (A-C) CBPI of all owners. (D-F) Individual trend values. (A) CBPI of all dogs. (B) Pain Severity Score (PSS) of all dogs. (C) Pain Interference Score (PIS) of all dogs. (D) Individual trend of CBPI. (E) Individual trend of PSS. (F) Individual trend of PIS. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ between each time point and day 0.

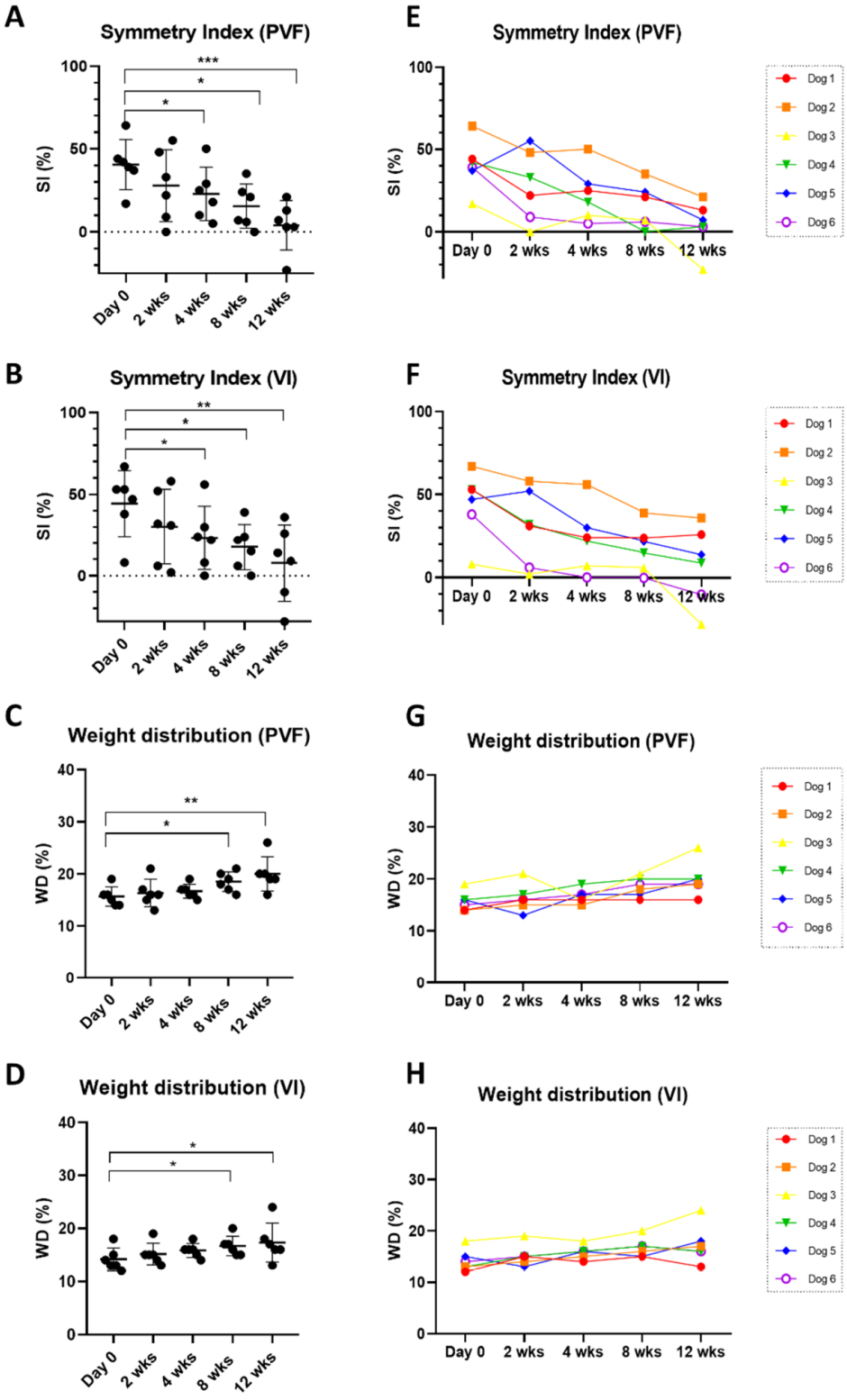

Fig. 4. Assessment of gait analysis. Data are presented as median and range. (A-D) Gait analysis of all dogs. (E-H) Individual trend values of gait analysis. (A) Symmetry index (SI) of peak vertical force (PVF). (B) SI of vertical impulse (VI). (C) Weight distribution (WD) of PVF. (D) WE of VI. (E) Individual SI trend of PVF. (F) Individual SI trend of VI. (G) Individual WD trend of PVF. (H) Individual trend of VI. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ between each time point and day 0.

mass of the proximal and distal hindlimb but also range of motion of the stifle joint were increased after injection. Because overexpressed TGF-β1 may lead to joint fibrosis and decreased range of motion in OA (25), inhibiting TGF-β1 by ELHLD peptides could prevent joint fibrosis and contribute to smooth motion in the joints of OA dogs.

We noted a significant improvement in the sum indexed CBPI scores from 4 weeks to 12 weeks after injection. All owners evaluated a noticeably lower CBPI score at 12 weeks after injection as compared with the score at preinjection. Among them, one owner scored their dog as 11 points in PSS at 12 weeks after injection, a result that indicates the dog did not suffer any pain from OA. Because pain is a hallmark of OA, this result can be interpreted as meaningful when evaluating the effectiveness of intra-articular injection of high-dose ELHLD. In addition, owners have the ability to observe their dogs for a much longer period of time and can identify their dog's subtle pain and behavioral changes in a familiar environment (2). For these reasons, the owner's assessment is indispensable for evaluating the drug response in animals. In this study, the CBPI score corresponded to the results of the orthopedic examination and gait analysis, which provides better support for improvement after intra-articular injection of high-dose ELHLD peptide.

In this study, we used kinetic gait analysis as an objective gold standard combined with other subjective outcomes. PVF and VI, which are the most often cited measures in gait analysis in veterinary medicine, were used and calculated as the SI and WD, respectively. The SI of PVF and VI were significantly improved at 4 weeks after injection, whereas the WD of PVF and VI significantly improved at 8 weeks postinjection. The difference in timing of improvement between the two parameters may suggest that significant improvement of asymmetry precedes a noticeable increase in weight bearing of the affected limb after intra-articular injection in dogs with OA. However, the SI results have greater support, because that measure is extensively used and generally recommended in canine kinetic gait analysis (14). In individual data, except for the aforementioned case, five dogs improved at 2 weeks after injection. Therefore, to provide more details regarding the timing of improvements in symmetry and WD after injection, an extension of this study using a larger sample size is warranted.

This study is the first to evaluate the efficacy of the ELHLD peptide in canine OA using kinetic gait analysis. Kinetic gait analysis is widely used in veterinary practice, as it can serve as an alternative to conventional gait assessment, which can reflect the observer's opinion or placebo effect (3). In this study, we used a pressure-sensitive walkway system for the gait analysis, as it presented high diagnostic sensitivity and specificity in a previous study (84.6% and 91.1%, respectively) (10,21). Twenty-nine subjects per group is the recommended sample size for a subjective assessment to be powered, but documented reports have determined that sufficient power is achieved with a small population when using kinetic gait analysis (8). To minimize the variability in the data, we provided a similar environment and velocity in each analysis. In addition, to standardize the comparisons, we calculated the PVF and VI into the SI and WD. Therefore, we obtained sufficient evidence with a small sample size that high-dose ELHLD peptide improves the clinical signs of canine OA, not only through subjective assessment but also through objective evaluation.

We found significant changes in several criteria, including orthopedic examinations, CBPI scores, and gait analyses, after high-dose ELHLD peptide injection, resulting in the improvement of six dogs with stifle OA. Although the limitations of this study include the variability associated with the small number of dogs and the single-group study design, there was a correspondence between the assessments of the degree and timing of improvement by the veterinarian and the owner. In addition, kinetic gait analysis also corresponded with these results, and we achieved a sufficient objective evaluation using a small sample size. Currently, there are only a few intra-articular medications that can be used for canine OA. We believe that this study provides preliminary evidence proving the clinical benefit of high-dose ELHLD peptide as an adjunctive or alternative treatment for canine OA.

In conclusion, all dogs with stifle OA were significantly improved in clinical signs after injection of high-dose ELHLD peptide. In present study, improvements were evaluated through the subjective assessment and objective analysis. The data obtained in this study provide a sufficient objective evaluation through kinetic gait analysis using a small sample size.

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References

1. Belshaw Z, Asher L, Dean RS. Systematic review of outcome measures reported in clinical canine osteoarthritis research. Vet Surg 2016; 45: 480-487.
2. Brønniche Møller Nielsen M, Pedersen T, Mouritzen A, Vitger AD, Nielsen LN, Poulsen HH, et al. Kinetic gait analysis in healthy dogs and dogs with osteoarthritis: an evaluation of precision and overlap performance of a pressure-sensitive walkway and the use of symmetry indices. PLoS One 2020; 15: e0243819.
3. Brown DC, Bell M, Rhodes L. 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. Am J Vet Res 2013; 74: 1467-1473.
4. Budsberg SC, Jevens DJ, Brown J, Foutz TL, DeCamp CE, Reece L. Evaluation of limb symmetry indices, using ground reaction forces in healthy dogs. Am J Vet Res 1993; 54: 1569-1574.
5. Cabon Q, Febre M, Gomez N, Cachon T, Pillard P, Carozzo C, et al. Long-term safety and efficacy of single or repeated intra-articular injection of allogeneic neonatal mesenchymal stromal cells for managing pain and lameness in moderate to severe canine osteoarthritis without anti-inflammatory pharmacological support: pilot clinical study. Front Vet Sci 2019; 6: 10.
6. Chevalier X. Intraarticular treatments for osteoarthritis: new perspectives. Curr Drug Targets 2010; 11: 546-560.
7. Cook JL, Smith PA, Bozynski CC, Kuroki K, Cook CR, Stoker AM, et al. Multiple injections of leukoreduced platelet rich plasma reduce pain and functional impairment in a canine model of ACL and meniscal deficiency. J Orthop Res 2016; 34: 607-615.
8. Fahie MA, Ortolano GA, Guercio V, Schaffer JA, Johnston G, Au J, et al. A randomized controlled trial of the efficacy of autologous platelet therapy for the treatment of osteoarthritis in dogs. J Am Vet Med Assoc 2013; 243: 1291-1297.
9. Finnson KW, Chi Y, Bou-Gharios G, Leask A, Philip A. TGF-β signaling in cartilage homeostasis and osteoarthritis. Front Biosci (Schol Ed) 2012; 4: 251-268.
10. Gibert S, Lequang T, Maitre P, Poujol L, Cachon T, Carozzo C, et al. Sensitivity and specificity to determine lameness in dogs with a pressure walkway system. Comput Methods Biomech Biomed Eng 2010; 13(Suppl 1): 61-62.
11. Gigante A, Callegari L. The role of intra-articular hyaluronan (Sinovial) in the treatment of osteoarthritis. Rheumatol Int 2011; 31: 427-444.
12. Henrotin Y, Sanchez C, Balligand M. Pharmaceutical and nutraceutical management of canine osteoarthritis: present and future perspectives. Vet J 2005; 170: 113-123.
13. Johnston SA. Osteoarthritis. Joint anatomy, physiology, and pathobiology. Vet Clin North Am Small Anim Pract 1997; 27: 699-723.
14. Keebaugh AE, Redman-Bentley D, Griffon DJ. Influence of leash side and handlers on pressure mat analysis of gait characteristics in small-breed dogs. J Am Vet Med Assoc 2015; 246: 1215-1221.
15. Kim HJ, Lee JW, Kwon YJ, Moon EJ, inventor; Ensol Biociences Inc., assignee. Novel Peptide and use thereof. European patent EP2588491B1. Sep 14, 2016.
16. Kim SE, Pozzi A, Yeh JC, Lopez-Velazquez M, Au Yong JA, Townsend S, et al. Intra-articular umbilical cord derived mesenchymal stem cell therapy for chronic elbow osteoarthritis in dogs: a double-blinded, placebo-controlled clinical trial. Front Vet Sci 2019; 6: 474.
17. KuKanich B, Bidgood T, Knesl O. Clinical pharmacology of nonsteroidal anti-inflammatory drugs in dogs. Vet Anaesth Analg 2012; 39: 69-90.
18. Lee MI, Kim JH, Kwak HH, Woo HM, Han JH, Yayon A, et al. 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. J Orthop Surg Res 2019; 14: 314.
19. Lories RJ, Luyten FP. The bone-cartilage unit in osteoarthritis. Nat Rev Rheumatol 2011; 7: 43-49.
20. Luna SP, Basílio AC, Steagall PV, Machado LP, Moutinho FQ, Takahira RK, et al. Evaluation of adverse effects of long-term oral administration of carprofen, etodolac, flunixin meglumine, ketoprofen, and meloxicam in dogs. Am J Vet Res 2007; 68: 258-264.
21. Maitre P, Arnault A, Verset M, Roger T, Viguier E. Chronic cranial cruciate ligament rupture in dog: four legs assessment with a walkway. Comput Methods Biomech Biomed Eng 2007; 10(Suppl 1): 111-112.
22. Mobasheri A, Henrotin Y. Identification, validation and qualification of biomarkers for osteoarthritis in humans and companion animals: mission for the next decade. Vet J 2010; 185: 95-97.
23. Moldovan F, Pelletier JP, Hambor J, Cloutier JM, Martel-Pelletier J. Collagenase-3 (matrix metalloprotease 13) is preferentially localized in the deep layer of human arthritic cartilage in situ: in vitro mimicking effect by transforming growth factor beta. Arthritis Rheum 1997; 40: 1653-1661.
24. Mu W, Xu B, Ma H, Ji B, Zhang Z, Li J, et al. Halofuginone attenuates articular cartilage degeneration by inhibition of elevated TGF-β1 signaling in articular cartilage in a rodent osteoarthritis model. Mol Med Rep 2017; 16: 7679-7684.
25. Neumann S, Lauenstein-Bosse S. Evaluation of transforming growth factor beta 1 in dogs with osteoarthritis. Open Vet J 2018; 8: 386-392.
26. Ping SH, Tian FM, Liu H, Sun Q, Shao LT, Lian QQ, et al. Raloxifene inhibits the overexpression of TGF-β1 in cartilage and regulates the metabolism of subchondral bone in rats with osteoporotic osteoarthritis. Bosn J Basic Med Sci 2021; 21: 284-293.
27. Pombo-Suarez M, Castaño-Oreja MT, Calaza M, Gomez-Reino J, Gonzalez A. Differential upregulation of the three transforming growth factor beta isoforms in human osteoarthritic cartilage. Ann Rheum Dis 2009; 68: 568-571.
28. Rédini F, Mauviel A, Pronost S, Loyau G, Pujol JP. Transforming growth factor beta exerts opposite effects from interleukin-1 beta on cultured rabbit articular chondrocytes through reduction of interleukin-1 receptor expression. Arthritis Rheum 1993; 36: 44-50.
29. van der Kraan PM. The changing role of TGFβ in healthy, ageing and osteoarthritic joints. Nat Rev Rheumatol 2017; 13: 155-163.
30. van der Kraan PM, Goumans MJ, Blaney Davidson E, ten Dijke P. Age-dependent alteration of TGF-β signalling in osteoarthritis. Cell Tissue Res 2012; 347: 257-265.

31. Waxman AS, Robinson DA, Evans RB, Hulse DA, Innes JF, Conzemius MG. Relationship between objective and subjective assessment of limb function in normal dogs with an experimentally induced lameness. Vet Surg 2008; 37: 241-246.
32. Webster RP, Anderson GI, Gearing DP. Canine Brief Pain Inventory scores for dogs with osteoarthritis before and after administration of a monoclonal antibody against nerve growth factor. Am J Vet Res 2014; 75: 532-535.
33. Wen ZH, Tang CC, Chang YC, Huang SY, Lin YY, Hsieh SP, et al. Calcitonin attenuates cartilage degeneration and nociception in an experimental rat model of osteoarthritis: role of TGF-β in chondrocytes. Sci Rep 2016; 6: 28862.
34. Yaeger PC, Masi TL, de Ortiz JL, Binette F, Tubo R, McPherson JM. Synergistic action of transforming growth factor-beta and insulin-like growth factor-I induces expression of type II collagen and aggrecan genes in adult human articular chondrocytes. Exp Cell Res 1997; 237: 318-325.
35. Zhang RK, Li GW, Zeng C, Lin CX, Huang LS, Huang GX, et al. Mechanical stress contributes to osteoarthritis development through the activation of transforming growth factor beta 1 (TGF-β1). Bone Joint Res 2018; 7: 587-594.
36. Zhen G, Cao X. Targeting TGFβ signaling in subchondral bone and articular cartilage homeostasis. Trends Pharmacol Sci 2014; 35: 227-236.