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FOR THE DEGREE OF MASTER

The Efficacy of Intra-articular Injection of a Selective Inhibitor of Smad1/5/8 pathway (Engedi 1000) for the Treatment of Osteoarthritis in Dogs

골관절염 개에서 선택적 **Smad**1/5/8 경로 억제제 (**Engedi** 1000)의 관절 내 주사 효과

by

Kyeonguk Choi

MAJOR IN VETERINARY CLINICAL SCIENCES

DEPARTMENT OF VETERINARY MEDICINE

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by
Kyeonguk Choi

Supervised by
Professor Oh-Kyeong Kweon

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지도교수 권 오 경

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Major in Veterinary Clinical Sciences, Department of Veterinary Medicine
Graduate School, Seoul National University

ABSTRACT

Transforming growth factor-β (TGF-β) plays an important role in the joints, causing osteoarthritic change in osteoarthritic joints. I investigated the efficacy of the intra-articular injection of the peptide drug Engedi 1000, which selectively inhibits the Smad1/5/8 pathway by inhibiting the action of TGF-β, for treating osteoarthritis (OA) in dogs. Forty-one client-owned dogs were divided into four treatment groups: Control, Engedi 1000, nonsteroidal anti-inflammatory drugs (NSAIDs), and NSAIDs + Engedi 1000. The effects of the Engedi 1000 were assessed via orthopedic examinations, owner assessment of outcomes, radiographic

examinations, and blood analysis. Orthopedic examination results show that the mean lameness and pain grades significantly improved after treatment in the Engedi 1000 group ($P < 0.05$) but not in the control group. The pain grades were significantly different between the Engedi 1000 and control groups at 4 and 12 weeks after treatment ($P < 0.05$). The NSAIDs + Engedi 1000 and NSAIDs groups both showed significant post-treatment improvements in the lameness and weight-bearing grades ($P < 0.05$), but only the NSAIDs + Engedi 1000 group showed significant post-treatment improvements in the pain grade ($P < 0.05$). The relative serum matrix metallopeptidase-13 levels were also significantly lower after treatment than before treatment in the Engedi 1000 group ($P < 0.01$). Moreover, no specific side effects were observed on radiographic examination or blood analysis when using Engedi 1000. These results show that Engedi 1000 has a therapeutic effect on OA in dog and has no specific adverse effects. In conclusion, intra-articular injection of Engedi 1000 could be an alternative treatment for OA in dogs.

Key words: osteoarthritis, peptide drug, Engedi 1000, intra-articular injection, dog

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CONTENTS

Introduction

Osteoarthritis (OA) is common joint disease in small animal. The prevalence of OA in primary-care veterinary practice in England is 6.6% (Dan *et. al* 2014). The prevalence of OA increases with age, 80% of dogs aged over 7 years show radiographic changes associated with osteoarthritis (Vérez-Fraguela *et. al* 2017). OA is a progressive joint disease and results in joint tissue degeneration, loss of function as well as pain (Innes 1995). OA causes joint deformity, restricted movement of the joint, articular cartilage damage, subchondral bone sclerosis, and formation of cysts and osteophytes. As a result, animals with OA have lameness, pain, and joint stiffness (Vérez-Fraguela *et. al* 2017).

Most cases of OA are managed with nutritional support, weight control, exercise management, physical therapy, and nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs reduce joint inflammation by inhibiting cyclooxygenase (COX); therefore, they are effective and widely used for treating osteoarthritis (Henrotin *et. al* 2005). However, since COX-1 modulates the homeostasis of normal cell function via prostaglandin synthesis, NSAIDs have adverse effects such as gastrointestinal toxicity and may cause liver and kidney damage. The renal side effects of NSAIDs increases in older animals (Vane *et. al* 1998). Therefore, there is an increasing need for safe alternatives that have a different mechanism from that of NSAIDs.

Transforming growth factor-β (TGF-β) is present in multicellular organisms and regulates many physiological functions (Newfeld *et. al* 1999). TGF-β

is known to play an important role in the joints (van der Kraan 2018). TGF-β signaling involves a heterometric complex composed of type I and II receptors. The type I receptor is called activin receptor-like kinase (ALK) (Graham *et. al* 2006). The functions of ALK1 and ALK5 are different in chondrocytes, depending on whether the Smad2/3 pathway or Smad1/5/8 pathway is activated (Finnson *et. al* 2008). In a normal healthy joint, active TGF-β signaling upregulates ALK5 to prevent the hypertrophy of chondrocytes through Smad2/3 phosphorylation, while it downregulates ALK1 related to the Smad1/5/8 pathway. However, in an osteoarthritic joint, the Smad1/5/8 pathway is activated through ALK1 by TGF-β, which results in chondrocyte hypertrophy (van der Kraan 2018). Moreover, the ratio of ALK1/ALK5 in the joint increases with age. Because ALK1 is associated with matrix metallopeptidase-13 (MMP-13), which destroys cartilage, and because ALK5 has an important role in aggrecan and type II collagen formation, which is important for a healthy joint, ALK1 signaling affects OA and causes age-related joint problems (Davidson *et. al* 2009). A recent study also showed that TGF-β levels in the synovial fluid of dogs with OA were higher than that of normal healthy dogs (Neumann *et. al* 2018). Therefore, it is assumed that blocking the Smad1/5/8 pathway by inhibiting the action of TGF-β or ALK1 may be helpful in treating OA. We developed a peptide drug Engedi 1000 or E1K (Ensol Bioscience, Daejeon, Korea) (Kim *et. al* 2014), which selectively inhibits the Smad1/5/8 pathway that leads to cartilage destruction and maintains the Smad2/3 pathway that keeps the cartilages healthy.

The objectives of this study were (1) to evaluate the effect of this peptide

drug on the clinical signs of OA in client-owned dogs, and (2) to provide safety data for this drug.

Materials and Methods

Animals

Forty-one client-owned dogs diagnosed with OA via orthopedic examination and radiography were enrolled in this study. This study was approved by the Institutional Animal Care and Use Committees of Seoul National University (SNU-180223-1). Informed consent was obtained from the owners prior to the dog`s enrollment. Dogs that underwent orthopedic surgery within the last 2 months, received systemic corticosteroid treatment within the past month, or received an intra-articular injection of corticosteroids within 3 months were excluded from the study. Forty-one dogs were divided into four groups. Ten dogs were treated with two intra-articular injections of 25 μg/kg Engedi 1000 (E1K group). Ten dogs were treated with NSAIDs medications for 4 weeks (NSAIDs group). Twelve dogs were treated with two intra-articular injections of 25 μg/kg Engedi 1000 and NSAIDs for 4 weeks (NSAIDs + E1K group). The remaining nine dogs were not given any medication or injection and formed the control group. All groups received physical therapy at each visit.

Orthopedic examination

Each dog was examined by one veterinarian (an author), who assessed the dog for lameness, weight-bearing, and pain before treatment and at 2, 4, 8, and 12 weeks after treatment. The lameness, weight-bearing, and pain were evaluated using grade (Tables 1, 2 and 3) (ROY *et. al* 1992).

Table 1. Lameness grade

Grade	
0	No lameness
1	Lameness after exercise Normal stance
2	Slight lameness when walking Normal stance
3	Moderate lameness when walking Normal or abnormal stance
4	Severe lameness when walking Abnormal stance
5	Reluctant to walk Reluctant to rise/stand

Table 2. Weight-bearing grade

Grade	
0	Full weight-bearing
1	Partial weight-bearing
2	Slight weight-bearing
3	Toe-touch weight-bearing
4	Intermittent weight-bearing
5	Non weight-bearing

Table 3. Pain grade

Grade	
0	None
1	Mild sign Dog turns head in recognition
2	Moderate sign Dog pulls limb away
3	Severs sign Dog vocalizes or becomes aggressive

Owner assessment of treatment outcomes

The evaluation of treatment outcome was based on a questionnaire submitted to the owners of the dogs involved in the study. The questionnaires were evaluated before treatment and at 2, 4, 8, and 12 weeks after treatment. The questionnaires included questions on the quality of life, activity, lameness, and stiffness, as well as questions on the therapeutic responses (Table 4).

Table 4. The questionnaire for owner`s assessment of treatment outcomes

Quality of life	
1	Normal
2	Somewhat uncomfortable
3	Pretty uncomfortable
4	Severely uncomfortable

Activity during the day	
1	Normal
2	Mildly reduced activity
3	Moderately reduced activity
4	Severely reduced activity

Stiffness after rest	
1	No
2	Mild
3	Moderate
4	Severe

Lameness during the day	
1	Normal
2	Mild 1) No pain in moving 2) Not uncomfortable when standing 3) Almost normal when climbing stairs and jumping 4) Mild pain on manipulating the joints
3	Moderate 1) Sometimes experiences pain when moving 2) Gets up slowly or prefers to sit rather than to get up 3) Reluctant to climb stairs or jump 4) Moderate pain on manipulating the joints
4	Severe 1) Pain when moving 2) Difficulty in standing up 3) Cannot climb stairs or jump 4) Severe pain on manipulating the joints

Response to treatment	
1	Excellent
2	Good
3	Fair
4	Bad

Radiographic examination

After the diagnosis of OA, radiographs of the osteoarthritic joint were acquired at the 4th and 12th week of treatment in the dogs in the E1K, NSAIDs, and NSAIDs + E1K groups. Two standard radiographs (craniocaudal and mediolateral) were viewed by one veterinarian to check for side effects such as increased inflammation or degenerative changes. In particular, in the stifle joint, the thickness of patellar ligament, the degree of joint effusion, and OA were evaluated. The degree of joint effusion was scored subjectively as follows: 0, normal; 1, mild; 2, moderate; and 3, severe (D'ANJOU *et. al* 2008). For the assessment of OA, a modification of the OA scores recommended by de Rooster and de Bruin was used (Table 5) (De Bruin *et. al* 2007).

Table 5. Osteoarthritis score in the stifle joint

Factor	Score : 0, normal; 1, mild; 2, moderate; 3, severe
1	Osteophyte formation at the proximal / distal edge of the patella
2	Subchondral sclerosis of the trochlear groove
3	Osteophyte formation on the fabella
4	Osteophyte formation in the long digital extensor muscle groove
5	Subchondral sclerosis of the tibial plateau
6	Osteophyte formation at the tibial attachment site of the cranial cruciate ligament
7	Osteophyte formation at the lateral and medial femoral condyle

Blood analysis and MMP-13 measurement

Blood samples of the treatment groups (E1K, NSAIDs, and NSAIDs + E1K) were collected from the jugular vein for determining the complete blood counts, serum biochemistry, electrolyte levels, and C-reactive protein (CRP) levels before treatment and at 4 and 12 weeks after treatment to check for systemic side effects. Residual serum samples were frozen at - 20℃, and these were used for measuring the serum MMP-13 concentration by using a canine MMP-13 enzyme-linked immunosorbent assay kit (MyBioSource, San Diego, USA).

Statistical analysis

Data were presented as the mean ± standard deviation (SD). Statistical evaluations were performed using IBM SPSS Statistics for Windows/Macintosh, Version 25.0 (IBM Corp., Armonk, USA). The normal distribution of the data was evaluated by the Shapiro-Wilk method. The one-way analysis of variance (ANOVA) was used to compare the population features of all the groups. A one-way ANOVA with repeated measures was used to evaluate the changes in the orthopedic examination results and owner assessment data over time. When a significant difference was observed, post-hoc analysis was performed using the Mann-Whitney U test and a sequentially rejective Bonferroni-Holm method. The changes in MMP-13 concentration and radiographic examination data before and after treatment were analyzed using a paired *t*-test. A P-value < 0.05 was considered significant.

Results

Cases

Details of the 41 dogs are summarized in Table 6. Among the dogs, 24 were male (1 intact; 23 castrated) and 17 were female (7 intact; 10 spayed). Their age ranged from 1 to 16 years (mean ± SD: 8.1 ± 4.5). Their body weight (BW) ranged from 2.27 to 80.5 kg (median ± SD: 5.8 ± 14.6). Significant differences in age were observed between the NSAIDs + E1K and control groups ($P < 0.01$) and between the NSAIDs + E1K and E1K groups ($P < 0.01$). No significant difference was observed in sex and BW among the four groups. No statistical difference was observed in the initial weight-bearing and pain grades among the four groups. However, the initial lameness grade between the NSAIDs + E1K and control groups showed a significant difference ($P < 0.01$).

One dog in the NSAIDs + E1K group and two dogs in the NSAIDs group were excluded because they underwent tibial plateau levelling osteotomy surgery during this study. Additionally, one dog in the NSAIDs group was excluded because its joint problem was due to immune-mediated arthritis.

Table 6. Data on the age, sex, BW, breed, orthopedic examination grade of the study population

	Control	E1K	NSAIDs + E1K	NSAIDs
n (41)	9	10	12	10
Age (years)	10.6± 4.2	10.7 ± 5.1 ##	4.5 ± 2.9 **	7.7 ± 3.1
Sex	MC (4), FS (4), IF (1)	MC (6), FS (2), IF (2)	MC (8), IM (1), FS (2), IF (1)	MC (5), FS (2), IF (3)
BW (kg)	4.7 ± 8.8	4.4 ± 3.8	6.4 ± 22.2	4.7 ± 10.4
Breed	Maltese (2) Pomeranian (1) Cocker Spaniel (1) Bichon Frises (1) Golden-Retriever (1) Poodle (1) Shih-Tzu (1) Yorkshire Terrier (1)	Maltese (7) Cocker Spaniel (2) Mixed (1)	Maltese (5) Doberman (1) Great pyrenees (1) Labrador Retriever (1) Old English bulldog (1) Pomeranian (1) Mixed (1) French bulldog (1)	Spitz (2) Maltese (2) Schnauzers (1) Pomeranian (1) Doberman (1) Cocker Spaniel (1) Bichon Frises (1) Chihuahua (1)
Lameness grade	1.8 ± 0.4	2.1 ± 0.5	2.8 ± 0.8 **	2.6 ± 0.8
Weight-bearing grade	0.3 ± 0.5	0.7 ± 0.6	1.6 ± 1.4	1.6 ± 1.3
Pain grade	0.9 ± 0.2	1.1 ± 0.4	1.3 ± 0.5	1.4 ± 0.7

All data are presented with the mean value ± SD, except for BW which is presented as the median value ± SD. Orthopedic examination grades are the pre-treatment grades. Significant difference compared to the control group (** $P < 0.01$), to the NSAIDs + E1K group ($^{\#\#}P < 0.01$).

BW, body weight; E1K, Engedi 1000; NSAIDs, nonsteroidal anti-inflammatory drugs; MC, castrated male; FS, spayed female; IF, intact female; IM, intact male

Orthopedic examination

The results of the orthopedic examinations obtained at each visit for the control and E1K groups are shown in Fig. 1, and those for the NSAIDs + E1K and NSAIDs groups are shown in Fig. 2. The lameness, weight-bearing, and pain grades of the E1K group decreased with time, while those of the control group remained similar over time, except for an increase in the weight-bearing grade. In the E1K group, the lameness grades significantly decreased at all time periods compared to the pre-treatment grade ($P < 0.05$), especially at the 4th week ($P < 0.01$). The weight-bearing grade showed a tendency to decrease but did not show a significant difference compared to the pre-treatment grade ($P > 0.05$). The pain grades significantly decreased at 4, 8, and 12 weeks when compared to the pre-treatment grade ($P < 0.05$), and significantly decreased at the 8th and 12th weeks compared to 2nd weeks ($P < 0.05$). A significant difference was observed in the pain grades at 4 and 12 weeks between the E1K and control groups ($P < 0.05$).

The lameness, weight-bearing, and pain grades of the NSAIDs + E1K and NSAIDs groups decreased with time. The NSAIDs + E1K group showed significant decreases in all orthopedic examination grades ($P < 0.05$), but the NSAIDs group did not show significant decreases in the pain grade. In addition, in the NSAID + E1K group, the lameness, weight-bearing and pain grades decreased significantly over time when compared to the grades at the 2nd week ($P < 0.05$).

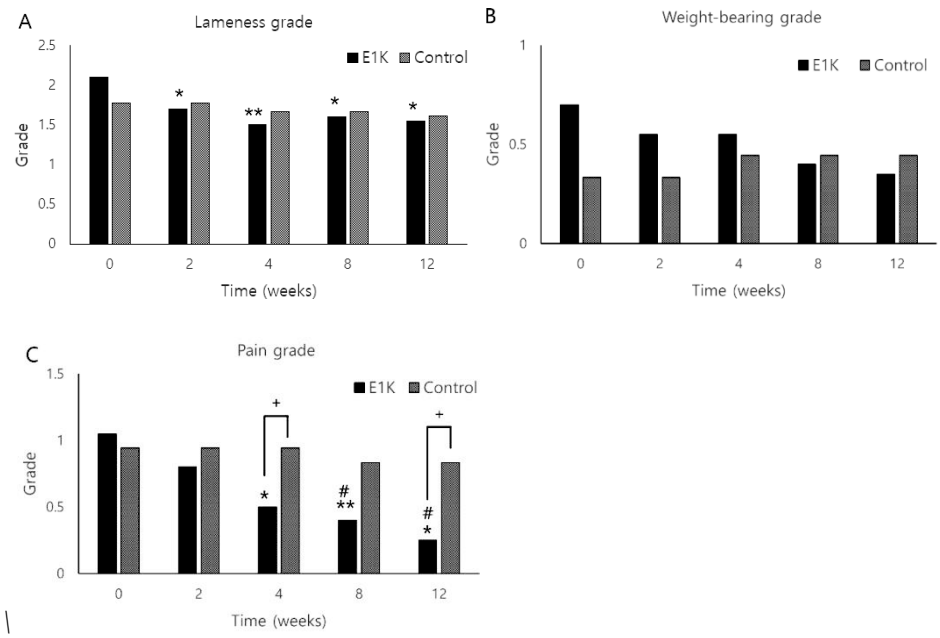

Fig 1. The mean orthopedic examination grades of the E1K and control groups before and at 2, 4, 8, and 12 weeks after treatment. (A) Lameness grade, (B) Weight-bearing grade, (C) Pain grade. The lameness and pain grades of the E1K group are significantly lower than the pre-treatment grades, but the weight-bearing grade of the E1K group is not. In the pain grade, a significant difference is observed between the control and E1K groups at 4 and 12 weeks. Significant difference compared to the pre-treatment grade (*$P < 0.05$, ** $P < 0.01$), to the grade at 2 weeks ($^{\#}P < 0.05$), and between the grades of the control and E1K groups $^{+}P < 0.05$). E1K, Engedi

1000

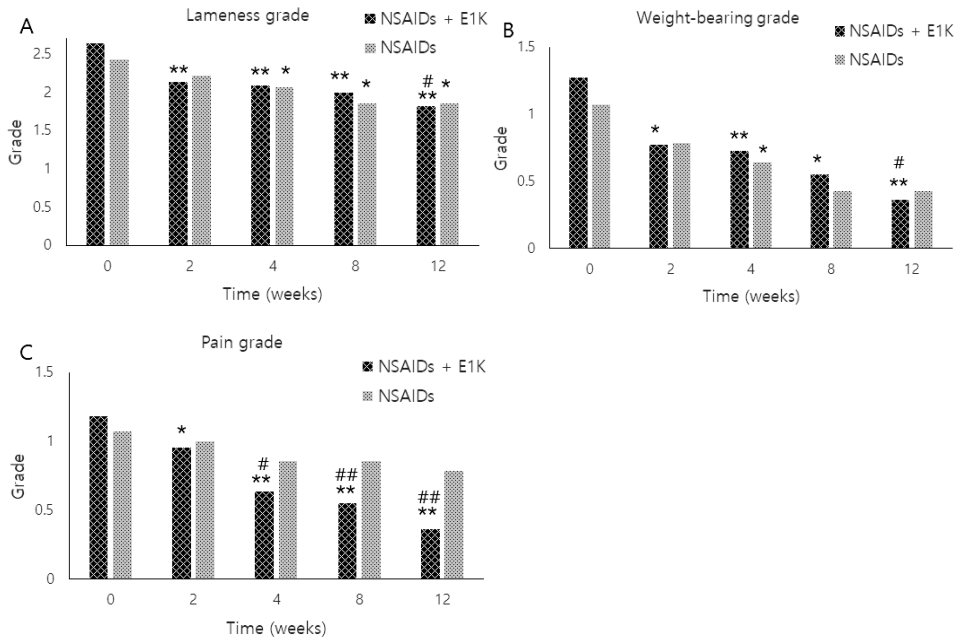

Fig 2. The mean orthopedic examination grades of the NSAIDs + E1K and NSAIDs groups before and at 2, 4, 8, and 12 weeks after treatment. (A) Lameness grade, (B) Weight-bearing grade, (C) Pain grade. The lameness and weight-bearing grades of both the groups are significantly lower than the pre-treatment grades, but the pain grade is significantly lower only in the NSAID + E1K group. Significant difference compared to the pre-treatment grade (*$P < 0.05$, ** $P < 0.01$) and to the grades at the 2nd weeks ($^{\#}P < 0.05$, $^{\#\#}P < 0.01$). E1K, Engedi 1000; NSAIDs, nonsteroidal anti-inflammatory drugs

Owner assessment of treatment outcomes

The results of owner assessment of the quality of life, activity, joint stiffness, and lameness obtained at each visit for the control and E1K groups are shown in Fig. 3. The results for the NSAIDs + E1K and NSAIDs groups are shown in Fig. 4. The mean pre-treatment scores of the E1K and control groups were 8 ± 2.1 and 7.6 ± 2, respectively. In the E1K group, the score showed a post-treatment decrease until the 4th week but showed a tendency to increase thereafter. However, the score at the 12th week was 7.6 ± 3.4, which was lower than the pre-treatment score. The control group showed a tendency of decrease in scores over time, but no significant difference was observed between the E1K and control groups.

The mean pre-treatment scores of the NSAIDs + E1K and NSAIDs groups were 10 ± 4 and 8.6 ± 1.6, respectively. In the NSAIDs + E1K group, the score decreased until the 12th week. In contrast, the NSAIDs group showed a tendency of increase at the 2nd week, then a decrease until the 8th week, and finally a re-increase at the 12th week. No significant difference was observed between the NSAIDs + E1K and NSAIDs groups.

The treatment responses assessed by the owners at every visit are shown in Table 7. All groups showed a tendency of increase in the ratio of ‘excellent’ responses. However, no significant difference was observed among the four groups ($P > 0.05$). On the basis of the analysis of the returned questionnaires, no adverse events were observed after the intra-articular injection of E1K, except for a slightly

decreased activity level for 2 days in one dog.

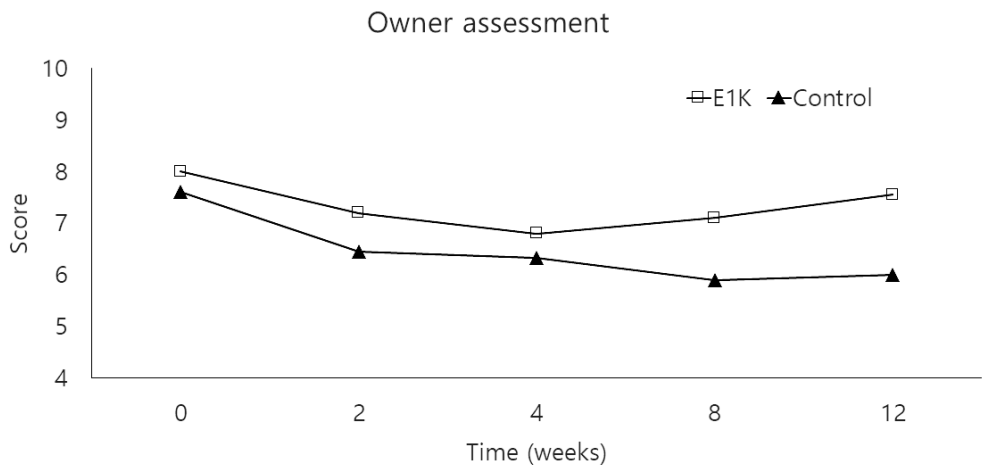

Fig 3. The average sum of the owners' assessments of the quality of life, activity, joint stiffness and lameness of their dogs in the control and E1K group. In both the groups, the scores at the 12th week are lower than the pre-treatment scores, but no significant difference is observed between the two groups. E1K, Engedi 1000

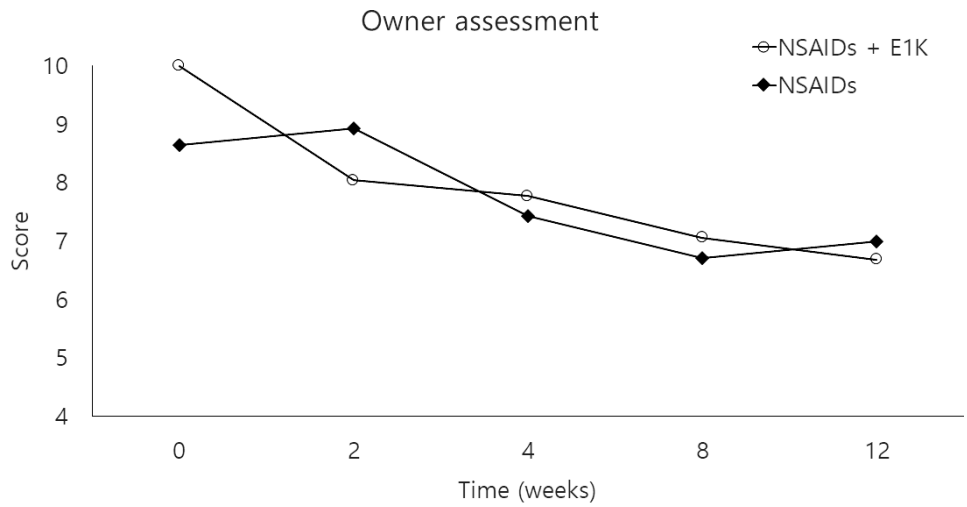

Fig 4. The average sum of the owners' assessments of the quality of life, activity, joint stiffness and lameness of their dogs in the NSAIDs + E1K and NSAIDs groups. In both the groups, the scores at the 12th week are lower than the pre-treatment scores, but no significant difference is observed between the two groups. E1K, Engedi 1000; NSAIDs, nonsteroidal anti-inflammatory drugs

Table 7. Owner satisfaction with treatment response

	Control (n)		E1K (n)		NSAIDs + E1K		NSAIDs	
After 2 weeks	Excellent	0	Excellent	3	Excellent	3	Excellent	3
	Good	7	Good	4	Good	5	Good	2
	Fair	2	Fair	3	Fair	3	Fair	1
	Bad	0	Bad	0	Bad	0	Bad	1
After 4 weeks	Excellent	1	Excellent	4	Excellent	2	Excellent	5
	Good	5	Good	3	Good	6	Good	2
	Fair	3	Fair	3	Fair	3	Fair	0
	Bad	0	Bad	0	Bad	0	Bad	0
After 8 weeks	Excellent	2	Excellent	5	Excellent	3	Excellent	6
	Good	5	Good	3	Good	4	Good	1
	Fair	2	Fair	1	Fair	4	Fair	0
	Bad	0	Bad	1	Bad	0	Bad	0
After 12 weeks	Excellent	3	Excellent	6	Excellent	4	Excellent	5
	Good	4	Good	1	Good	5	Good	2
	Fair	2	Fair	2	Fair	2	Fair	0
	Bad	0	Bad	1	Bad	0	Bad	0

E1K, Engedi 1000; NSAIDs, nonsteroidal anti-inflammatory drugs

Radiographic examination

Radiographic examinations revealed OA of the stifle joint in 8 dogs in the E1K group, 10 in the NSAID + E1K group, and 7 in the NSAID group and the degree of OA, joint effusion, and thickness of the patellar ligament were evaluated in those dogs (Fig 5). In the E1K group, the mean OA score remained unchanged at 6.0 ± 5.8. The mean joint effusion score was as follows: pre-treatment = 1.6 ± 0.7; after 4 weeks = 1.5 ± 0.9; and after 12 weeks = 1.6 ± 0.8. The mean thickness of the patellar ligament was as follows: pre-treatment = 2.4 ± 0.6 mm; after 4 weeks = 2.4 ± 0.5 mm; and after 12 weeks = 2.4 ± 0.5 mm. In the NSAIDs + E1K group, the mean OA score was as follows: pre-treatment = 6.0 ± 4.5; after 4 weeks = 6.0 ± 4.5; and after 12 weeks = 6.4 ± 4.6. The mean joint effusion score was as follows: pre-treatment = 2.4 ± 0.6; after 4 weeks = 2.3 ± 0.6; and after 12 weeks = 2.6 ± 0.6. The mean thickness of the patellar ligament was as follows: pre-treatment = 2.7 ± 0.7 mm; after 4 weeks = 2.7 ± 0.7 mm; and after 12 weeks = 2.6 ± 0.8 mm. In the NSAIDs group, the mean OA score was as follows: pre-treatment = 7.2 ± 4.4; after 4 weeks = 7.2 ± 4.4; and after 12 weeks = 7.7 ± 4.5. The mean joint effusion score was as follows: pre-treatment = 1.9 ± 0.8; after 4 weeks = 1.8 ± 0.9; and after 12 weeks = 1.8 ± 1. The mean thickness of the patellar ligament was as follows: pre-treatment = 2.3 ± 0.5 mm; after 4 weeks = 2.3 ± 0.5 mm; and after 12 weeks = 2.2 ± 0.6 mm. No significant difference was observed in the OA score, degree of joint effusion, and thickness of the patellar ligament before treatment and at the 4th and 12th weeks of treatment in each group and among the three groups. Two dogs in the E1K group

and one dog in the NSAIDs + E1K group had OA of the elbow joint. These dogs also showed no specific post-treatment changes.

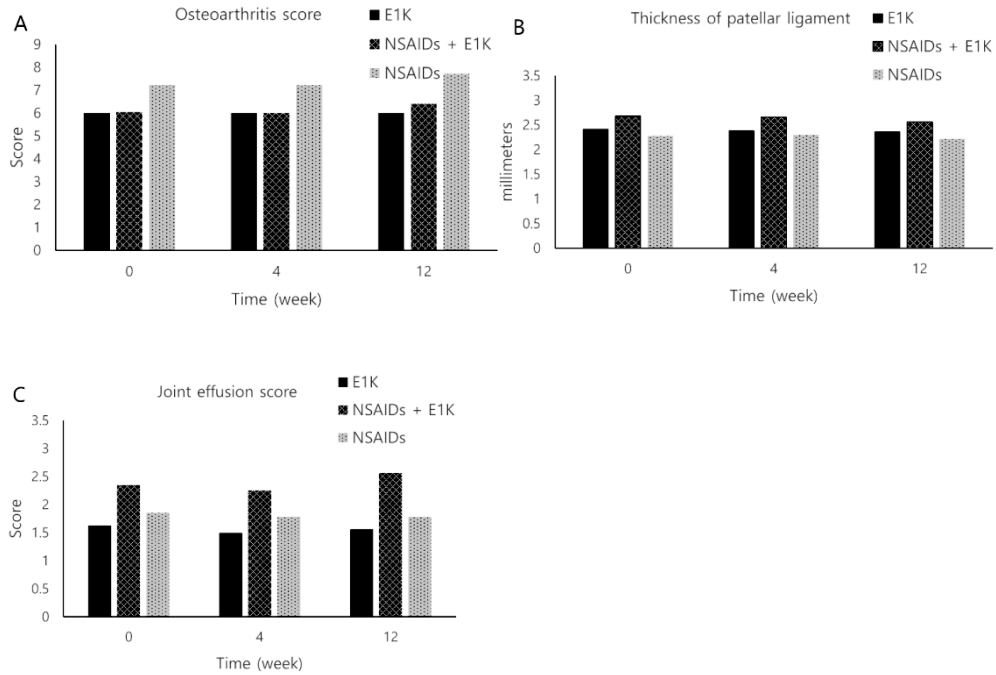

Fig 5. The mean OA grade, join effusion score, and thickness of the patellar ligament of the E1K, NSAIDs + E1K, and NSAIDs groups before treatment and at 4 and 12 weeks after treatment. (A) OA score, (B) joint effusion score, and (C) thickness of the patellar ligament do not significantly differ over time. E1K, Engedi 1000; NSAIDs, nonsteroidal anti-inflammatory drugs

Blood analysis and MMP-13 measurement

In the E1K group, one dog with otitis externa, otitis media, epidermal cyst, and a skin mass had CRP levels above the normal range before treatment and at 4 weeks (38.5 and 46 mg/dL, respectively). However, at 12 weeks, the CRP level returned to the normal range at 15.3 mg/dL. The CRP levels of one dog in the NSAIDs + E1K group were 35.1, 118.6, and 18.7 mg/dL before treatment, at 4 week, and at 12 weeks, respectively. One dog in the NSAIDs group also showed a CRP level above the normal range (35.3 mg/dL) only at 4 weeks. Except for these three dogs, none showed clinically significant abnormal values in complete blood counts, serum biochemistry, electrolyte levels, and CRP levels.

The relative serum MMP-13 levels of the E1K group were significantly lower at 12 weeks than before treatment ($P < 0.01$; Fig. 6).

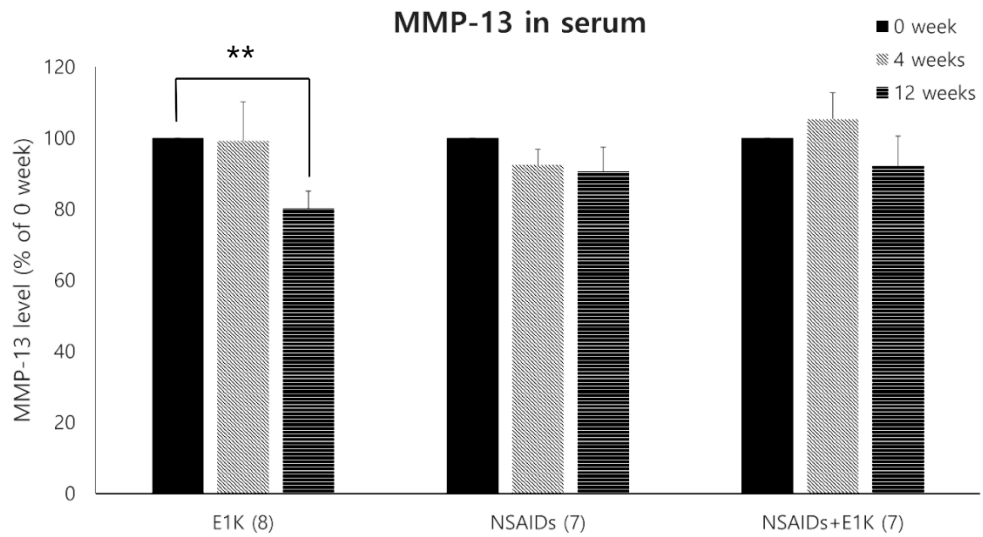

Fig 6. MMP-13 level before treatment and at 4 and 12 weeks after treatment in the three groups. The graph and bars depict the means ± SD, as determined by the percent relative to the pre-treatment MMP-13 level. Significant difference compared to pre-treatment (** $P < 0.01$). MMP-13, matrix metallopeptidase-13; E1K, Engedi 1000; NSAIDs, nonsteroidal anti-inflammatory drugs

Discussion

In this study, I explored the effectiveness and safety of a peptide drug (E1K), which is a selective inhibitor of Smad1/5/8 pathway by inhibiting the action of TGF-β, for the treatment of OA in dogs. Experimental dog models of OA, such as the cranial cruciate ligament transection model, are known to develop OA similar to spontaneous OA (Adams *et. al* 1983, McDevitt *et. al* 1977, McDevitt *et. al* 1976). However, the pathological change in the cartilages of the experimental model was different from that of spontaneous OA in dogs (Liu *et. al* 2003). For evaluating the therapeutic effect of E1K on naturally occurring OA in dogs, I performed this study in a real clinical setting on client-owned dogs with OA. Therefore, I could evaluate the therapeutic effect of E1K on naturally occurring OA in dogs, not experimental models.

Since this study was the first clinical study on the therapeutic effects of E1K on OA, E1K was applied to dogs which had chronic and not severe symptoms rather than acute severe symptoms. Dogs that were older and with chronic symptoms were classified into the control and E1K groups. In dogs with severe acute clinical symptoms, NSAIDs—the standard treatment for osteoarthritis—were applied, and they were compared with dogs treated with the combination of NSAIDs and E1K to confirm the efficacy of the E1K treatment. As a result, the dogs in the control and E1K groups were relatively older and significantly older than the dogs in the NSAIDs + E1K group. In the orthopedic examination, the E1K group showed better

improvement than did the control group, showing significant differences, especially in the pain grade. The NSAIDs + E1K group also showed better improvement than did the NSAIDs group, but without a significant difference. These orthopedic examination results showed that E1K was particularly effective in dogs with chronic OA.

The joint tissue expresses the *MMP* genes, and their expression levels are generally low. The activity of MMP is inhibited by the tissue inhibitors of MMPs (TIMPs) (Dean *et. al* 1987). Chondrocytes produce both MMPs and TIMPs, and their balance is crucial to the homeostasis of cartilage differentiation and growth in the normal healthy joint (Howell 1986). However, in OA joints, synovitis is observed, and it plays an important role in pain, joint inflammation, and cartilage degradation via the production of MMPs (Sutton *et. al* 2009). The levels of activated MMPs are higher in OA joints than in normal joints (Brama *et. al* 2000, Clegg *et. al* 1997). Therefore, attempts to treat OA by using drugs that inhibit MMPs have continued in human and veterinary medicine for reducing joint damage, osteochondral angiogenesis, and pain (Baragi *et. al* 2009, Mapp *et. al* 2010). Because TGF-β induces the transcription of *MMP* genes, E1K that inhibits TGF-β in OA joints ultimately reduces the production of MMP-13 (Martel-Pelletier *et. al* 2012). In addition, while MMP inhibitors might produce side effects such as joint stiffness, joint fibroplasia, anemia, and liver enzyme elevation, this study showed that E1K did not produce any musculoskeletal and systemic side effects (Martel-Pelletier *et. al* 2012, Rao 2005, Renkiewicz *et. al* 2003).

Osteoarthritic joint tissue produces nerve growth factor (NGF) (Driscoll *et. al* 2016, Pecchi *et. al* 2014, Seidel *et. al* 2010). NGF is upregulated in the cartilage during pain perception, and it plays roles in pain transmission and hyperalgesia, as well as in inducing synovial cell proliferation (Driscoll *et. al* 2016, Raychaudhuri *et. al* 2009). The use of NGF antibody to treat OA pain in humans has been investigated (Brown *et. al* 2013, Brown *et. al* 2012, Hefti *et. al* 2006, Iannone *et. al* 2002). It reduced the OA pain and improved joint function; however, it produced side effect such as paresthesia and accelerated the development of OA, especially in patients receiving NSAIDs therapy (Brown *et. al* 2010, Brown *et. al* 2012, Lane *et. al* 2010). NGF production is stimulated by many growth factors, and recent studies showed that TGF-β induced NGF production in chondrocytes more potently than did interleukin-1β (Davidson *et. al* 2015, Iannone *et. al* 2015, Seidel *et. al* 2010, Sellam *et. al* 2010). E1K was also thought to reduce NGF production in osteoarthritic joints by inhibiting TGF-β. Moreover, E1K had not adverse effects in dogs receiving concomitant NSAIDs therapy in this study. These adverse effects of NGF antibody seemed similar to drug-induced osteonecrosis (Martel-Pelletier *et. al* 2012). However, radiographic examinations of the joints in this study did not reveal any evidence of osteonecrosis, and the use of E1K seemed unrelated to the NGF antibody side effects. Therefore, the downregulation of MMP and NGF by E1K is considered to play an important role in joint pain relief and may result in improvement of clinical symptoms.

A previous in vitro study revealed that E1K regenerates damaged cartilages;

therefore, I hypothesized that E1K treatment would decrease osteoarthritic change and this would be observed on radiographic examination in the E1K-treated group (Kim *et. al* 2014). However, radiographic examinations revealed that although the clinical symptoms improved after treatment, the changes were not significant. As the presence of OA on radiographic examination did not correlate with animal limb function, the improvement in clinical symptoms would not have been visible on radiography (Gordon *et. al* 2003). The reduction in joint effusion did not appear significant with E1K treatment, but no significant decrease was observed in the group treated with NSAIDs either. Therefore, the degree of joint effusion on radiography and the clinical symptoms were unlikely to be significantly related.

The owner satisfaction with treatment response in all groups was better after treatment, with an increase in the ratio of 'excellent' responses. The owners' assessments of the quality of life, activity, joint stiffness, and lameness of their dogs showed post-treatment improvements in all treatment groups. Although a comparison of the E1K and control groups showed that the E1K group had improved limb function, this result was not consistent with the owners' assessment. Since the real limb function of dogs and owners' perceptions of their dogs' limb function were not always consistent, we considered this result a discrepancy (Burton *et. al* 2009, Suwankong *et. al* 2007).

This study had several limitations. First, a quantitative evaluation of lameness was not performed, and only orthopedic examinations were conducted. Kinetic and kinematic gait analyses would provide more accurate results. Second,

the therapeutic effect according to the injection intervals of E1K was limited, because this study design included only two injections in week 0 and 2. Different injection intervals would help establish the therapeutic duration and appropriate injection intervals for E1K. Finally, because the role of TGF-β was complex and its activity differed according to the joint status, I had to decide which OA grade would be appropriate to perform E1K injection in and which timing would be the most effective (Iannone *et. al* 2003, Neumann *et. al* 2018, van der Kraan 2018).

In conclusion, intra-articular injection of Engedi 1000 improved the clinical signs and reduced serum MMP-13 levels in dogs with OA. Engedi 1000 injection produced no specific radiological changes or adverse effects. Accordingly, I suggest that intra-articular injection of Engedi 1000 could be an alternative treatment for OA in dogs.

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국문 초록

골관절염 개에서 선택적 Smad1/5/8 경로 억제제 (Engedi 1000)의 관절 내 주사 효과

지도교수 권 오 경

최 경 욱

서울대학교 대학원

수의학과 임상수의학 전공

TGF-β는 정상 관절의 항상성을 유지하는데 중요한 역할을 하며 골관절염이 있는 관절에서 골관절염이 생기게 하는 변화를 만든다. 본 연구에서는 골관절염이 있는 개에서 TGF-β를 억제하여 Smad1/5/8 경로

를 선택적으로 억제하는 펩타이드 약물인 Engedi 1000을 관절 내로 주입하여 골관절염이 생기는 변화를 억제함으로써 치료 효과가 나타나는지 살펴보았다. 보호자가 있는 41마리의 개를 대상으로 네 군으로 나누었다: 대조군, Engedi 1000 처치군, 비스테로이드성 진통소염제 처치군, 비스테로이드성 진통소염제와 Engedi 1000을 같이 처치한 군. Engedi 1000의 골관절염에 대한 치료 효과는 정형검사와 보호자의 치료 반응에 대한 평가, 영상학적 검사와 혈액검사를 통해 평가하였다. 정형검사에서 Engedi 1000을 처치한 군은 치료 전에 비해 파행도와 통증의 개선이 유의적으로 나타났으나 대조군에서는 그렇지 않았다. 또한 특히 통증부분에서는 두 군 간의 유의적인 차이를 보였다. 비스테로이드성 진통소염제와 Engedi 1000을 같이 처치한 군과 비스테로이드성 진통소염제를 처치한 군에서도 치료 전에 비해 파행도와 부중도의 개선이 유의적으로 나타났으나 통증부분에서는 비스테로이드성 진통소염제와 Engedi 1000을 같이 처치한 군에서만 유의적인 차이가 나타났다. Engedi 1000을 처치한 군에서는 혈청 matrix metallopeptidase−13의 수치가 치료 전에 비해 치료 후에 유의적으로 감소함을 확인하였다. 영상검사 및 혈액검사에서 Engedi 1000을 사용한 경우에 특이적인 부작용이 보이지는 않았다. 이러한 결과들을 종합해 볼 때 본 연구에서는 Engedi 1000이 골관절염이 있는 개에서 치료 효과가 있으며 특별히 큰 부작용이 없다고 평가하였다. 결론적으로 Engedi 1000의 관절 내 주사는 골관절염이 있는 개에서 하나의

치료적 대안이 될 수 있을 것이다.

주요어: 골관절염, 펩타이드 약물, Engedi 1000, 관절 내 주사, 개

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