Original Article

Chondroprotective efficacy of undenatured collagen type II on canine osteoarthritis secondary to medial patellar luxation

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Abstract

Osteoarthritis (OA) is found secondary to medial patellar luxation (MPL) which is one of the most common diseases in small-breed dogs. Management of the disease is surgical correction or medical treatment. In patients who are unable to do the surgical correction, non-steroidal anti-inflammatory drugs (NSAIDs) remain the drugs of choice for OA therapy. Nowadays nutraceuticals have become more popular since there are no side effects when given for a long period. The purpose of this study was to evaluate the clinical chondroprotective effects of undenatured collagen type-II (UCII) on osteoarthritis secondary to canine medial patellar luxation. Nine small-breed dogs (thirteen stifle joints) received UCII for 120 days. The results revealed a statistically significant difference between before and after receiving UCII in CBPI (p-value <0.05), ultrasonographic scores and joint effusion. In contrast, there was no significant difference in lameness score, radiographic examination and blood chemistry. In conclusion, UCII may be an alternative treatment for OA nutraceutical chondroprotective products which could be a co-treatment with the MPL treatment as UCII can improve the clinical signs in some dogs, which could not be managed by surgical treatment.

Keywords: chondroprotective effect, dog, medial patellar luxation, osteoarthritis, undenatured collagen type II

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Introduction

Osteoarthritis (OA) is a degenerative joint disease which is characterized by degenerative articular cartilage and formation of new bone in the affected joint surfaces which also causes changes in the synovial membrane. Osteoarthritis is a slow progressive inflammatory disease causing lameness, decreased weight-bearing capacity and occasional joint effusion (Vaughan-Scott and Taylor, 1997; Alam *et al*., 2011a; Alam *et al*., 2011b).

The degeneration of articular cartilage is an important point to detect OA in its early stages. The most common diagnostic technique is radiography which cannot reveal the changes in articular cartilage or the synovial membrane. Recently in human studies, the ultrasonography technique was recommended for detection of early stage OA by detecting abnormalities in the articular cartilage surface. Moreover, this technique is desirable as a quick and easy screening method for diagnosis of human knee osteoarthritis with its non-invasiveness and cost-effectiveness (Okano *et al*., 2016).

Management of osteoarthritis is most often by conservative methods with multimodals to control pain and clinical signs for a better quality of life and to slow the progress of OA by giving Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) combined with nutraceuticals and weight and exercise management. NSAIDs remain the drug of choice for OA therapy. Nevertheless, NSAIDs cannot be given continuously for a long period of time due to their adverse effects (Brosseau *et al*., 2003).

Nutraceuticals have become more popular since there are no side effects when given for a long period of time. One of the nutraceuticals that can be used to slow the progress of OA is undenatured collagen type II. Undenatured collagen type II is a glycoprotein from chicken sternum (Bagchi *et al*., 2002), which can decrease inflammation and results in reducing pain for osteoarthritis patients (Deparle *et al*., 2005; Bagi *et al*., 2017). Therefore, this study aims to investigate the chondroprotective effects of undenatured collagen type II on dogs with OA secondary to medial patellar luxation.

Materials and Methods

Animals: Small breed dogs with a lameness problem were diagnosed and those with medial patellar luxation (severity grade I-III) were selected for the study group. The animals included in this study had to be more than 2 years of age and have no other diseases. Only animals with a body condition score 3-6/9 were selected for the study. The evaluation of body condition score scale was based on the Nine-Interger BSC scale system (Laflamme, 1997). All dogs must not have been treated for MPL and not take NSAIDs or any pain killers at the time they were in the study. The animals had to be fed only commercial standard food. The owners of the dogs had to have informed consent and permission for the study.

Study designs: The 21 stifle joints from 13 dogs were treated with undenatured collagen type II (UCII) at the recommended dose of 10 mg per animal once a day for 16 weeks. The animals were evaluated in both stifles for clinical outcome before treatment at day 0 (D0) and post-treatment program at week 2 (W2), week 4 (W4), week 8 (W8), week 12 (W12) and week 16 (W16). The clinical evaluations included ultrasonographic findings, radiographic findings, lameness scores, blood profiles and owner questionnaires. On each visit, the patients were assessed for monitoring all the clinical evaluations except blood profiles which were examined on D 0 and W 16 for any adverse effects and health status. The study was completed at the end of W16.

The study was approved by the Chulalongkorn University Animal Care and Use Committee (CU-ACUC) Bangkok, Thailand. The approved number is 1931008.

- Lameness score

The gait of the animals was evaluated weekly by walking and trotting from lameness score criteria modified from (Impellizeri *et al*., 2000).

The data was collected by taking videos for reviewing the score. The lameness score criteria began from 0 to 5 points (from normal gait when walking and trotting to non-weight when walking and trotting.

- Radiographic examination

A conventional radiography of both stifle joints was taken from two views: mediolateral and caudocranial views to evaluate the osteoarthritis score. This osteoarthritis score was based on the OA grading system of Wessely *et al*. (2017).

The radiographic score was examined from mediolateral and caudocranial views to evaluate the osteoarthritis score. Fifteen locations of each stifles were evaluated for the OA grading system, each location was graded on a numeric scale from 1 to 4 as none to severe

- Blood profile

The blood test was assessed at D 0 before the study and W 16 at the end of study to evaluate health status and any adverse effects. Blood samples were collected from either cephalic or saphenous veins to evaluate the hematological profile and serum biochemistry. The blood sample was separated into two equal portions. The hematological profile was evaluated using an ethylenediamine tetra acetic acid (EDTA) tube while serum biochemistry was evaluated using a heparin tube.

- Ultrasonographic examination

Ultrasonography was used to evaluate stifle joint alterations with real-time 7.5-MHz linear (Kramer *et al*., 1999; Ramirez-Flores *et al*., 2017). Stifle joints were examined following the acoustic approach of Kramer *et al*., (1999). The ultrasound evaluation was analyzed base on the ultrasound scoring system of Goranov *et al*., (2013). Synovial fluid, articular cartilage and bone surface of the femoral condyles scores were evaluated from 4 parts of the femoral condyles; the proximal and distal of both medial and distal of both medial and lateral areas. The individual scores range was between 0 and 11 points. Furthermore, the articular cartilage

thickness of the femoral condyles was recorded in millimeters.

Ultrasonographic scores evaluation was modified from Goranov *et al*., (2013) which was divided into three sections;

Section 1; the synovial fluid score was calculated from 0-4 points by evaluated joint fluid appearance. If normal, the appearance of joint fluid and the anechoic content (black) appearance in ultrasound, the score would be increased by the appearance of joint fluid, echogenicity, and heterogeneity.

Section 2; the articular cartilage score was calculated from 0-3 points by evaluation from cartilage of femoral condyle which is typically anechoic content (black) appearance of the hyaline cartilage with asmooth margin in ultrasound. The score increased by heterogeneous appearance of the hyaline cartilage with irregular margins.

Section 3; the bone surface score was calculated from 0-4 points by evaluating the bone surface of femoral condyle which is usually absent irregular and/or rounded interruptions of the hyperechoic boundary of bone (white) appearance in ultrasound. The score increased by irregular and/or rounded interruptions of the hyperechoic boundary of bone appearance.

- ***Owner questionnaires***

The owners were assessed using the Canine Brief Pain Inventory (Canine BPI) questionnaire by the University of Pennsylvania to detect the severity of osteoarthritis pain (Cleeland, 2006).

Statistical analysis: The ultrasound scoring system, articular cartilage thickness of the femoral condyles, osteoarthritis scoring system, lameness scores, pain scores and quality of life scores from owner's questionnaires were evaluated using One-way anova. The blood profile was evaluated using paired T-test. All statistical analysis was performed using Prism program version7. *P-value* < 0.05 was considered as statistically significant.

Results

In this study, 13 dogs (21 stifle joints) were examined for 16 weeks. However, one dog suddenly died and other systemic diseases were observed in three dogs during the study. Therefore, only 13 stifles were studied over the 16 weeks period of study. From the total sample observed, 61.5% were Pomeranian, 23% were Chihuahua and 15.5% were Yorkshire terriers. Among 9 dogs, there was six male dogs (45.15%) and three females (53.85%). Mean ± SD age and body weight of this study group was 7 ± 4.5 and 3.35 ± 1.043 kg respectively. The severity of medial patellar luxation divided into MPL grade1 (7.7%), MPL grade 2 (30.77%) and MPL grade 3 (61.53%).

Blood profiles: In this study the blood sample was collected from the left forelimb for the complete blood count (CBC) and serum chemistry before starting the experiment (day0; D 0) and after the experiment (week 16; W 16), to assess the health status of the patients pre and post treatment including the effect of collagen type II on the serum chemistry. The results revealed a non-significant difference with p-value >0.05 between D0 and W16 exception blood urea nitrogen (BUN) was p-value <0.05.

Lameness score: All of the dogs had been evaluated 6 times within 16 weeks. The results of the lameness score had no significant difference (p-value =0.337; p-value> 0.05) in each exam week. However, some dogs with both left and right medial patellar luxation revealed an obvious improvement of both legs' lameness score.

Ultrasonographic examination: In the present study, ultrasonographic score, synovial fluid score, articular cartilage score, bone surface scores and cartilage thickness were analyzed. The data was collected six times within sixteen weeks and the result is shown as Mean±SD in table 1.

Significant difference was observed between D 0 and W 8 as well as D 0 and W 16 while there was no difference in other weeks examined. From the examinations, the most obvious lesion of articular cartilage was found at the medial ridge of the femoral condyle while it was not obvious at the lateral ridge.

The result of the assessment of the synovial fluids (Mean ± SD) is shown in Figure 1. Significant difference between D 0 (pre-treatment) to W 2 to the others (P-value <0.05) was observed.

Regarding the articular cartilage score, slight improvement was observed between D 0 and W 16 in seven stifle joints from thirteen. However, no significant difference was observed in any examinations. Mean ± SD of proximal medial cartilage scores are shown in figure 2. The treatment found that cartilage thickness had no significant differences (p-value>0.05) in each examination. Mean ± SD of cartilage thickness are revealed in table 2.

Radiographic examination: The stifle joints were assessed for osteoarthritis scores (OA scores) in two standards of the position in radiography. OA scores were evaluated in a range; 0-45. No significant differences (p-value>0.05) were found in any examination. Figure 3 showed a mean ± SD of this data.
Owner questionnaires: The severity of pain and quality of life was significantly different (p-value<0.05) between D 0 andW 16.

Discussion

UCII had been used to control pain, reduce inflammation, promote healthy joints and improve joint mobility as well as increasing flexibility in arthritis cases (Deparle *et al*., 2005; Bagi *et al*., 2017). The ability of a mucosal immune system to actively inhibit the systemic immune response to feed antigens had been used as a therapy for some chronic inflammatory and autoimmune conditions (C Crowley *et al*., 2009). In this study, it was revealed that age, gender, weight, breed and severity of MPL were not the main factors which affected the results of the study. There were four excluded dogs in this research because one had died and the other three had got systemic problems which

had to be treated by other drugs. The data of these dogs was not used for analysis.

The blood profiles of all dogs between pre and post-treatment were not significantly different. Interestingly, the mean of blood urea nitrogen (BUN) had slightly decreased however it was in the normal range in both pre and post treatment. D'Altilio *et al*., (2007) also found CBC (RBC, hemoglobin, hematocrit, platelet, WBC, neutrophils, eosinophils, basophils, lymphocytes and monocytes) and serum chemistry (creatinine, ALT and ALP) results as well.

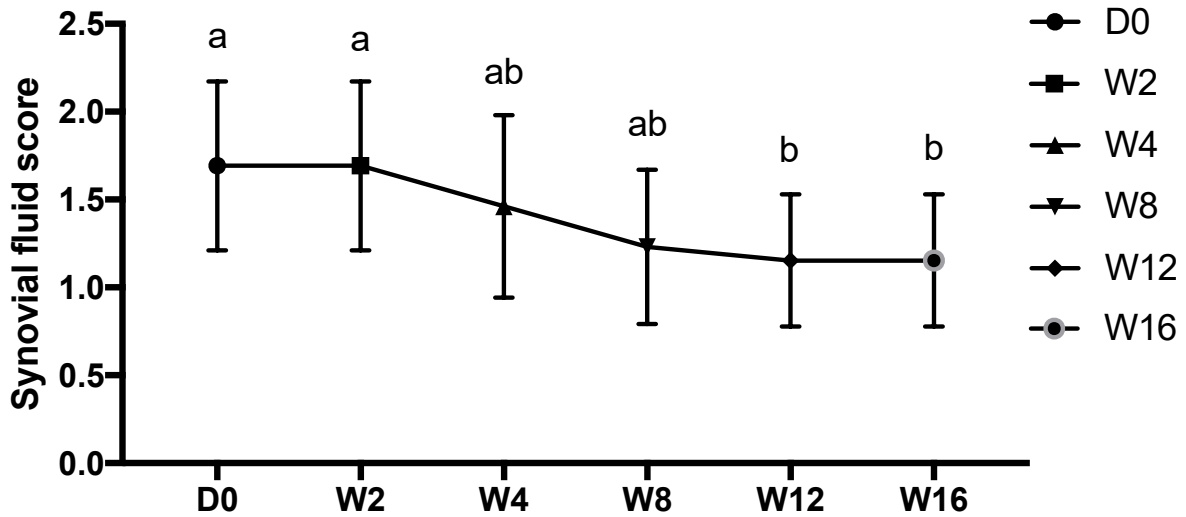
Table 1 Mean±SD of ultrasonographic, synovial fluid, articular cartilage and bone surface score in every examined week

Ultrasonographic parameters		Treatment group					
		Day 0	Week 2	Week 4	Week 8	Week 12	Week 16
Ultrasonographic score		5.846±1.345[a]	5.231±1.092[a]	4.923±1.115[a]	4.615±1.557[b]	4.308±1.109[b]	3.692±1.251[b]
Synovial fluid score		1.692±0.480[a]	1.692±0.480[a]	1.462±0.518[b]	1.231±0.438[b]	1.154±0.375[b]	1.154±0.375[b]
Articular cartilage score	Proximal aspect of medial femoral condyle	1.692±0.480	1.385±0.506	1.385±0.506	1.308±0.480	1.231±0.438	1.077±0.640
	Distal aspect of medial femoral condyle	1.385±0.506	1.231±0.438	1.077±0.493	1.154±0.688	1.077±0.493	0.923±0.640
Bone surface score	Proximal aspect of medial femoral condyle	0.769±0.599	0.769±0.599	0.769±0.599	0.769±0.599	0.692±0.630	0.384±0.506
	Distal aspect of medial femoral condyle	0.307±0.480	0.153±0.375	0.230±0.438	0.153±0.375	0.153±0.375	0.153±0.375

a, b - values with different superscript letters for each variable in the same row are significantly different (p <0.05)

Table 2 Mean±SD of cartilage thickness in every examined week

Groups	Positions	Day0	Week2	Week 4	Week 8	Week 12	Week 16
Treatment group	Medial	0.196±0.059	0.198±0.057	0.200±0.057	0.192±0.049	0.207±0.064	0.196±0.043
	Middle	0.192±0.057	0.188±0.050	0.192±0.049	0.186±0.042	0.2±0.054	0.180±0.043
	Lateral	0.234±0.080	0.226±0.083	0.218±0.064	0.223±0.072	0.230±0.063	0.207±0.049



a, b - values with different superscript letters for each variable in the same row are significantly different (p <0.05)

Figure 1 Kaplan-Meier survival curves for showing different median survival time of cats with arterial with different categories including age (A), breeds (B), concentration of circulating glucose (C) and concentration of creatinine (D). The two curves differ significantly at p<0.05.

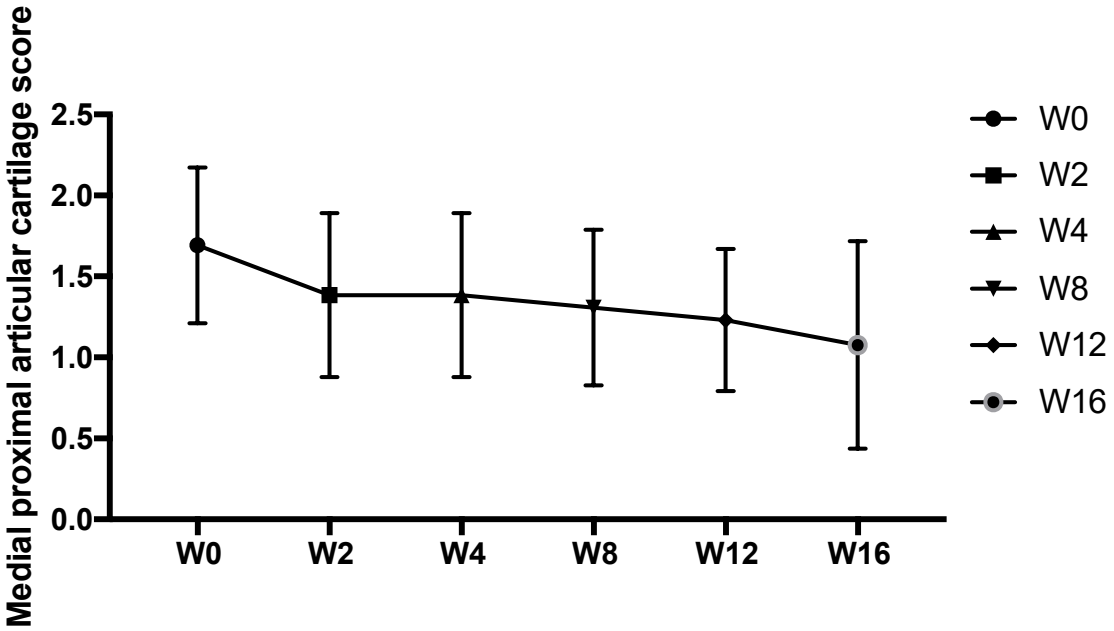


Figure 2 The mean ± SD of proximal medial articular cartilage score in collected weeks

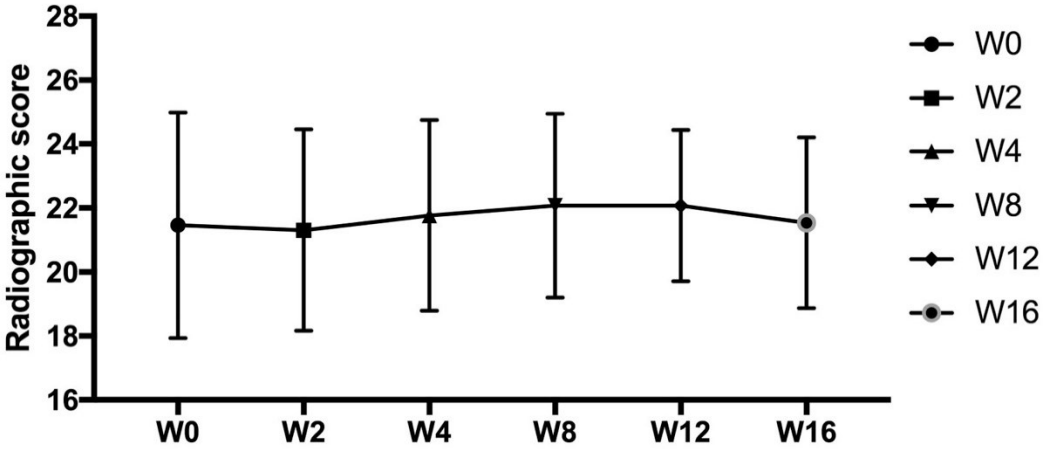


Figure 3 The mean ± SD of radiographic score in each monitoring week

The result of the lameness score in this study was not significantly different between pre and post-treatment. The lameness score was verified by blind technique. In addition, most patients had a pre-treatment lameness score at 1-2 out of 5, thus the lameness score of post-treatment was not different within the group. Improvement was found in a few of the high lameness score dogs. The result in this study were different from the previous study by *D'Altilio et al*., (2007) which found a significant decrease in overall pain and pain associated with lameness and limb manipulation. The reason for disagreement from the D'Altilio *et al*., (2007) study might be the assessment method using DeParle *et al*., (2005) criteria which graded the pain score from 0-10 while this present study evaluated by walking and trotting from the lameness score criteria of Impellizeri *et al*., (2000).

The results from CBPI questionnaire found that the evaluation of pain and quality of life had significantly improved between pre and post- treatment. However, Sathienbumrungkit (2018) found that there was a significant difference within the placebo group which indicated the placebo effect. The placebo effect and conservative management should be considered in further study for more accurate results.

Osteoarthritis scores (OA scores) from the radiographic examination showed no significant difference between pre and post-treatment. This could be because the OA in medial patellar luxation was the result of a low-grade inflammation and the early stage of OA development in the affected canine stifle (Camber, 2017; Di Dona *et al*., 2018). Alam *et al*., (2006) found that the early stages of OA were difficult to diagnose using radiography.

The results of the ultrasonography scores were composed of synovial fluid, medial and lateral proximal and distal articular cartilage and bone surface. Evaluated total scores differed significantly. When these scores were assessed separately, we found only the synovial fluid score had a significant difference between pre and post treatment. The main erosion area of the femoral condyle in this study was on the medial proximal condyle ridge which corresponded with the previous report from Jahrupatrakorn (2017). The assessment of medial proximal condyle revealed slight improvement

nevertheless it showed no significant difference. The articular cartilage thickness from the transverse view of the ultrasound revealed no significant differences in medial, middle and lateral condyle. Bagchi *et al*., (2002) found that type II collagen was the structural protein which can be found in cartilage and it was responsible for tensile strength and toughness. Undenatured collagen type II is a precursor of collagen produced in joints, however, it cannot be used directly as a joint promoter because the provided collagen is broken down into small molecules which stimulate cytokines for decreasing inflammation and regulation of immunity. Deparle *et al*., (2005) mentioned that UCII worked in down regulating the autoimmune response in the case of rheumatoid arthritis. For osteoarthritis patient, UCII could help in the reduction of inflammation and pain relief. Furthermore, UCII has the ability to stop the immune system from attacking and damaging its own joint cartilage. Hence, UCII tends to give an obviously better outcome which reduces joint damage in immune mediated osteoarthritis such as rheumatoid. This type of collagen increases immunotolerance. Small doses of UCII prevent attack by T killer cells. The pathogenesis of osteoarthritis is not related to immune-mediated mechanisms. Osteoarthritis resembles rheumatoid arthritis in the form of cartilage damage and inflammatory response. Thus, this research studied osteoarthritis in dogs in contrast to the previously referred to examination. In addition, the dogs in this study which had osteoarthritis did not show clearly different results between before and after treatment.

For further study, the author suggests using other practical instruments that will decrease the effect of other factors and provide a more accurate outcome. For example; Computerized Tomography Scan (CT scan), Magnetic Resonance Imaging (MRI), histopathology, synovial analysis, force plate gait analysis or C-reactive protein test. All of the mentioned appliances may give a better and more reliable outcome than radiographic examination or ultrasonographic scores. Moreover, the sample size should be increased due to some samples being excluded from the criteria in the clinical trial study.

In conclusion, from the present study, UCII could improve the chondroprotective effect in the treatment of osteoarthritis that was secondary to medial patellar luxation though it was not obviously different. This may be due to the mechanisms that UCII mainly effects in osteoarthritis associated with immune response which may not involve osteoarthritis from the present study. Nevertheless, there are some beneficial aspects to using undenatured collagen type II such as inflammation improvement from the result of joint effusion, CBPI and impression. Therefore, this nutraceutical substance may not directly support MPL dogs but it could be used as an alternative treatment especially in MPL dogs which cannot be managed by a surgical treatment.

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