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Clinical Studies

Evaluation of Polysulfated Glycosaminoglycan Administered by Aqua-acupuncture Injection for Treatment of Canine Osteoarthritis: A Randomized, Controlled, Blinded Clinical Study

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ABSTRACT

Intramuscular administration of polysulfated glycosaminoglycan (PSGAG) reduces joint inflammation and pain, and has demonstrated efficacy in the treatment of canine osteoarthritis. This study sought to determine whether injection of PSGAG using aqua-acupuncture (aqua-AP) technique is more effective than standard intramuscular (IM) treatment. Arthritic canines were randomly assigned to Control Group (IM) or Test Group (aqua-AP) injections. All subjects received a commercial FDA-approved PSGAG dosed at 2 mg/lb (4.4 mg/kg) twice weekly for four weeks. Outcomes were compared between groups using scores from Canine Brief Pain Inventory (CBPI) and Quality of Life (QoL) completed by the owners (blinded to treatment group). Twenty-eight patients completed the study (12 controls, 16 test dogs). Evaluation of CBPI pain severity score improvement showed that test dogs had significant improvement (p=0.0004) achieved after 1 week of treatment and the improvement remained significant throughout the course of the trial. Controls also improved with significance attained at Week 3 (p=0.047). For CBPI pain interference score, the Test Group again had significant improvement (p=0.002) after 1 week of treatment, and improvement remained significant throughout the 4-week trial. Controls also had significant improvement, starting slightly later at Week 2 (p=0.025) and continuing through Week 4. Both groups demonstrated improvement for QoL score, with statistical significance starting from Week 2 for aqua-AP test dogs (p=0.011) and from Week 3 for controls (p=0.004). The results from this study suggest PSGAG administration using aqua-AP injection technique is non-inferior and potentially more effective and efficient than standard IM injection in mitigating pain severity for canine osteoarthritis patients.

Keywords: Adequan®, aquapuncture, Canine Brief Pain Inventory, canine osteoarthritis, polysulfated glycosaminoglycans, quality of life

ABBREVIATIONS: aqua-AP: aqua-acupuncture; CBPI: Canine Brief Pain Inventory; DMOAD: disease-modifying osteoarthritis drug; IM: intramuscular; NSAID: non-steroidal anti-inflammatory drug; OA: osteoarthritis; PCP: pharmacopuncture; PSGAG: polysulfated glycosaminoglycans; QoL: quality of life

Osteoarthritis (OA) is a common clinical problem in the canine population. The disease can be characterized as inflammation of synovial joints resulting in damage and loss of articular cartilage, which can result in bone injury. Clinically the most significant signs of OA are lameness and stiffness. Canine OA can occur in any breed or age, although it is more common in large breed and geriatric individuals. Obesity is a significant predisposing factor.[1]

While there is no cure, OA treatments aim to control pain while slowing further deterioration of the joints, and to restore normal function to the greatest degree possible. Multi-modal approaches tailored to each individual patient are considered ideal. Pharmacologic and nonpharmacologic treatment options for management of acute and chronic pain are extensive. They can include non-steroidal anti-inflammatory drugs (NSAIDs), opioids, and local anesthetics which provide pharmacologic analgesia; whereas non-pharmaceutical, non-surgery treatments encompass weight management, therapeutic exercise, hydrotherapy, photobiomodulation, electromagnetic field therapy, nutraceuticals, and acupuncture.[2,3]

Aqua-acupuncture (aquapuncture, aqua-AP) is a modified acupuncture technique which involves the injection of fluids and soluble product into designated acupuncture points.[4] Although mentioned in some ancient Chinese texts, the first aqua-AP injections recorded were in China

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in the 1950s.[4] When aqua-AP is used for the injection of pharmaceuticals, it is also called pharmacopuncture (PCP). Aqua-acupuncture aims to strengthen and lengthen stimulation of an acupuncture point.[4] Some interesting investigations into the mechanism of action of aqua-AP suggest the technique provides a longer stimulation of acupoints via pressure and/or changes in the spatial configuration of acupoint tissue matrix, which activate neuronal signaling. This is coupled with the effect of the injected substrate, with different substrates having varied degrees of stimulation. One study investigated the magnitude of substrate effect by identifying the extent of activated neurons expressing Fos protein (biomarker for neuronal activation) in the dorsal horn of the spinal cord.[5]

A variety of injectables can be used for aqua-AP, including vitamin B_{12}, diluted vitamin B_{12} with sterile saline, local anesthetics, sedatives, herbal medicine extracts, bee venom, acepromazine, and vaccines. Perdrizet et al. showed that aqua-AP administration of canine distemper vaccination resulted in a significantly elevated humoral immune response compared to conventional vaccine administration methods.[6] The polysulfated glycosaminoglycan (PSGAG) injectable[a] used in this study is a disease-modifying osteoarthritis drug (DMOAD) approved by the FDA for the treatment of degenerative or traumatic arthritis. The medication has been shown to move quickly into joints binding to components of the cartilage matrix which slows further joint degradation, decreasing pain and inflammation. Reported side effects include inhibition of hemostasis (dose related) and diarrhea.[7-9]

The objective of this study was to determine if the efficacy of the PSGAG injectable solution can be improved by using aqua-AP injection technique compared to the standard intramuscular (IM) injection for treating canine osteoarthritis. The hypothesis was that OA dogs treated with PSGAG injected into acupuncture points would have greater improvement of OA associated pain and quality of life (QoL) than those treated with standard PSGAG intramuscular injection.

MATERIALS AND METHODS

Subject Population and Study Design

The subject population consisted of arthritic canine patients regardless of age, breed, or sex. Patients were recruited from a clinic of five veterinarians, who were all informed of the protocol and encouraged to recruit patients. Owners of the potential participants were provided an information packet, which contained a cover sheet, a client consent form, frequently asked questions sheet, a Canine Brief Pain Inventory (CBPI) scoring sheet, and a PSGAG[a] informational pamphlet. Patients whose owners consented to participation in the study and met the following inclusion criteria were enrolled in the study: (1) a lameness score of 2 or greater (Table 1); (2) pain on manipulation of the joint and/or a decreased range of motion; (3) without known systemic disease that compromised their general health; (4) without any neurologic related gait changes; and (5) did not receive NSAID treatment within 10 days of start of study protocol or corticosteroids within 30 days.[10] During the study, owners of enrolled dogs agreed that there would be no new medications/supplements added or changes made in the diet or medications/supplements that their dogs were receiving at the start of the study.

Qualified subjects were randomly assigned to either the Control Group or Test Group. Subjects in the Control Group received the standard procedure of IM injection of PSGAG, whereas those in the Test Group received the same solution but with aquapuncture injection technique. Each random group assignment was determined based on a token drawn from a container filled with 25 tokens for the Control Group (marked "C") and another 25 tokens for the Test Group (marked "T"), as a total number of 50 subjects was the desired sample size when planning the study.

Table 1: Clinical lameness scoring system used for assessing study dogs[10]

Lameness Grade	Clinical Evaluation
1	Walks normally
2	Slightly lame when walking
3	Moderately lame when walking
4	Severely lame when walking
5	Reluctant to rise and will not walk more than 5 paces

Experimental Procedure

At clinic presentation each owner remained in the car during the visit, which was easily executed due to the clinic's COVID-19 pandemic protocol which was strictly imposed during the time period of this study. A veterinarian admitted the patient into the clinic for a body weight measurement and treatment. The dog was then returned to the owner after the treatment.

Patient treatment protocol for the Control Group used the standard PSGAG intramuscular injection protocol given at the standard dose of 2 mg/lb (4.4 mg/kg) twice a week for four weeks. Treatment was administered by a veterinarian, with assistance of a technician providing restraint. The IM injections were given in the epaxial muscles (3 cc syringe, 3/4 inch 23-gauge needle) away from acupuncture points to avoid accidental acupoint stimulation. Alternate sides were used at each treatment session.

The treatment protocol for the Test Group used the same PSGAG dose as the controls (based on body weight), but the solution was equally divided and injected into 4 acupuncture points (right and left, BL-11 and BL-23). A tuberculin syringe with a 5/8 inch 25-gauge needle was used for the injection with a new needle used for each acupoint. The acupuncture points BL-11 (Influential point for bone) and BL-23 (Back-*shu* Association point for Kidney) were selected for aquapuncture using PSGAG, as these acupoints are commonly used for treatment of canine arthritis. The BL-11 acupoint is located at the cranial edge of the scapula 1.5 *cun* lateral to the dorsal spinous process of T1, and BL-23 is located on the dorsal lateral aspect of the spine 1.5 *cun* lateral to the caudal border of the dorsal spinous process of L2 (Figure 1).[4] All treatments in the study were performed by the author who is a certified veterinary acupuncturist (CVA).

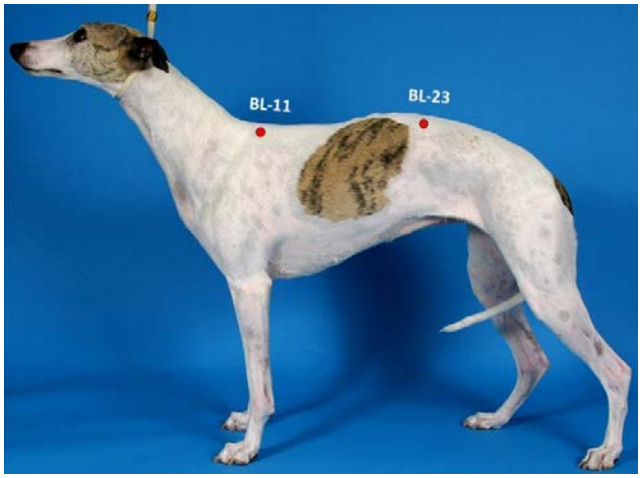

Figure 1: Location of acupuncture points BL-11 and BL-23

Outcome Data and Statistical Analysis

The Canine Brief Pain Inventory was used as the primary outcome measurement for the study. It is a validated quantitative pain measurement assessment that has been shown to be effective for the evaluation of chronic pain related to canine osteoarthritis and bone cancer.[11-13] The CBPI is modeled after the validated assessment of pain severity and function interference, Brief Pain Inventory scoring in humans.[13] In the CBPI, the pain severity area has 4 measurements, each on a 0 to 10 rating (0=no pain and 10=severe pain). The interference domain has six areas evaluated by the owner that interfere with daily activities, also on a 0 to 10 scale (0=no interference and 10=completely interferes). An overall pain severity score was calculated by averaging the four pain indices. Similarly, an overall pain interference score was calculated from the six interference areas.[11,13,14] Each subject's owner, who stayed in the car during the treatment and hence was blinded to the group assignment, was asked to complete the CBPI form (for the previous week) while the patient was receiving treatment in an exam room. To enhance the reliability of the survey for each subject, the same person, who was usually the primary caregiver, was asked to complete the weekly questionnaire.

In addition, a global quality of life (QoL) questionnaire, which evaluates overall assessment on a 1 to 5 scale from poor to excellent, was completed by the owners one week prior to the start of the study to serve as a baseline. The second evaluation (week 1 response) was done just before the 2nd week treatment started. This allowed time after the completion of each week's treatment for the owner to evaluate the patient's response.[13]

A commercial software[b] was used for all statistical analyses. All outcome data described were considered numerical and presented with summary statistics mean±SD. Two-factor repeated measure ANOVA using the ranked data was applied to test the overall group difference in measurement improvement. To compare between groups at each assessment time, nonparametric Wilcoxon Rank Sum test was applied. The improvement within each group at each assessment time was assessed by the Wilcoxon Signed Rank test. A significance level of 0.05 was used to determine statistical significance. Assuming a 75% probability that an outcome in the Test Group is better than one in the Control Group, a sample size of 25 subjects in each group was planned, which would offer a power of approximately 90% to reject the null hypothesis with a 0.05 significance level.[15]

RESULTS

Subject Population Signalment

A total of 29 canine patients diagnosed with arthritis and met the inclusion/exclusion criteria were enrolled in this randomized controlled clinical study. Difficulties in recruitment resulted in a short fall of enrolled patients even after recruitment extended for a period of 15 months. The random assignment procedure resulted in 13 subjects being assigned to the Control Group and 16 subjects being assigned to the Test Group. One subject in the Control Group withdrew due to diarrhea starting the day after the first treatment. The total subject number included in the data analysis was therefore 28, with 12 in the Control Group and 16 in the Test Group. No significant differences between groups were found in subject signalment data, including sex proportion, age, and body weight (Table 2). Both groups consisted of a wide variety of breeds: Control Group - Belgian Tervuren, St Bernard, Great Dane, Lhasa Apso, German Shepard dog, English Bulldog, Labrador/Golden Retriever mix, Golden Retriever, Pit Bull/Labrador mix, Labrador; Test Group - Pit Bull mix, Great Dane, Labrador Retriever, Jack Russell, Terrier Lab-mix, Golden Retriever mix, Golden Retriever, English Setter, Rhodesian Ridgeback, Mixed-breed, Golden Retriever, Bernese Mountain dog.

Table 2: Summary statistics of subject signalment data

	Control (n=12)	**Test** (n=16)	***p*-value**
Sex (Female %)	41.7% (5/12)	62.5% (10/16)	0.445
Age (mean±SD; years)	10.45±1.98 [6.5-13.0]	10.47±2.53 [5.8-16.3]	0.741
Weight (mean±SD; lbs)	85.25±33.83 [30.0-137.0]	68.63±29.04 [11.0-130.0]	0.156

Both study groups had similar limb and joint OA prevalence (Table 3). Among the 12 control subjects, 8 (66.7%) had hindlimbs only affected and the other 4 dogs (33.3%) had both fore and hindlimbs affected. Among the 16 dogs in the Test Group, 10 (62.5%) had hindlimbs only affected while the other 6 (37.5%) had both fore and hindlimbs affected. None of the subjects had forelimbs only affected by OA. Hip disease was common in the 28 dogs enrolled in this study with the majority of dogs having at least one hip joint affected: 10 (83.3%) in the Control Group and 14 (87.5%) in the Test Group. When all dogs in the study with an affected

Table 3: Summary statistics of subject's affected limb and joint data

Limb or Joint Affected with OA	Control Group (n=12)	Test Group (n=16)	Control vs. Test (*p*-value)
Hindlimb Only	8 (66.7%)	10 (62.5%)	1.000
Both Fore and Hindlimb	4 (33.3%)	6 (37.5%)	1.000
Forelimb Only	0 (0%)	0 (0%)	1.000
Hip	10 (83.3%)	14 (87.5%)	1.000
Stifle	1 (8.3%)	4 (25.0%)	0.355
Elbow	2 (16.7%)	4 (25.0%)	0.673
Shoulder	2 (16.7%)	1 (6.25%)	0.560

OA=osteoarthritis; tan shade=limb, green shade=joint

hip joint (85.7%) were compared with non-hip OA dogs (14.3%), there was statistical significance (p=0.047) suggestive of hip OA being prevalent in the population of dogs participating in the present study. One (8.3%) control subject and 4 (25%) in the Test Group had a stifle joint affected; 2 (16.7%) in the controls and 4 (25%) in the Test Group had an elbow joint affected, and 2 (16.7%) controls and only 1 (6.25%) in the Test Group had an affected shoulder joint. Either for the affected limbs or for affected joints, the group difference in the proportions was not statistically significant (Table 3).

CBPI Pain Severity Score

The mean CBPI pain severity scores within each treatment group over time (pre-treatment Week 0 and 4 weekly post-treatment assessments) were calculated (Figure 2, Table 4). At Week 0, the CBPI pain severity score in the Control Group was 16.58±8.54 and was 18.81±7.09 in the Test Group. Statistically, the difference was not significant (p=0.90), which suggests group comparability for pre-treatment pain severity condition.

At Week 1, the Control Group's CBPI pain severity score slightly reduced to 16.42±9.02 (improvement= 0.17±4.37, p=0.627), and further reduced to 14.83±9.55 (improvement=1.75±7.34, p=0.371) in Week 2, 11.58±8.15 (improvement=5.00±7.12, p=0.047) in Week 3, and 10.92±11.28 (improvement=5.67±10.41, p=0.072) in Week 4. Statistical significance was demonstrated for pain score improvement at the Week 3 assessment, compared to the Week 0 score. The score reduction at Week 4, despite having a greater mean value than in Week 3, was not statistically significant due to the greater variation among the subjects.

For the Test Group, at Week 1, the CBPI pain severity score reduced significantly to 13.81±8.14 (improvement= 5.00±4.65, p=0.0004), and further reduced to 11.41±8.46 (improvement=7.38±5.35, p=0.0002) in Week 2, 9.75±6.80 (improvement=9.06±6.04, p<0.0001) in Week 3, but had a slight increase to 10.31±8.39 (improvement=8.50±6.61, p=0.0002) in Week 4. This suggests that significant improvement on pain severity score was achieved after 1 week of treatment, and the improvement remained significant throughout the course of the trial.

Table 4: Severity score summary statistics (mean±SD) from Canine Brief Pain Inventory filled out by owners of study dogs in the Control Group and Test Group during conduct of the study

	Week 0	Week 1	Week 2	Week 3	Week 4
Control	Severity Score	Severity Score	Severity Score	Severity Score	Severity Score
	16.58±8.54	16.42±9.02	14.83±9.55	11.58±8.15	10.92±11.28
	Improvement from Week 0				
	—	0.17±4.37 p_1=0.627	1.75±7.34 p_1=0.371	5.00±7.12 p_1=0.047*	5.67±10.41 p_1=0.072
% improvement compared to baseline at start of study		1.03% improvement	10.6% improvement	30.2% improvement	34.2% improvement
Test	Severity Score	Severity Score	Severity Score	Severity Score	Severity Score
	18.81±7.09	13.81±8.14	11.41±8.46	9.75±6.80	10.31±8.39
	Improvement from Week 0				
	—	5.00±4.65 p_1=0.0004***	7.38±5.35 p_1=0.0002***	9.06±6.04 p_1<0.0001***	8.50±6.61 p_1=0.0002***
% improvement compared to baseline at start of study		26.6% improvement	39.2% improvement	48.2% improvement	45.2% improvement
Group Comparison		p_2=0.010*	p_2=0.030*	p_2=0.208	p_2=0.591

p_1=p-value of improvement compared to Week 0; p_2=p-value of improvement comparison between groups; * p<0.05; ** p<0.01, *** p<0.001

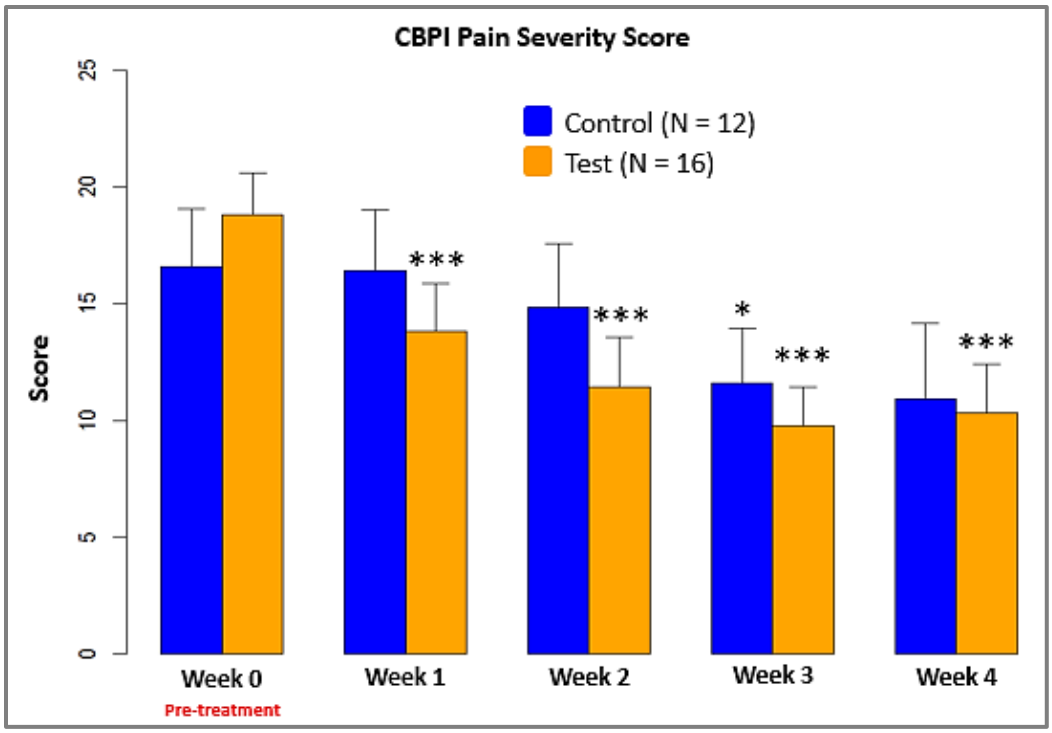

Figure 2: Mean (with standard error) CBPI pain severity score within each treatment group over time (pre-treatment Week 0 and 4 weekly post-treatment assessments). * p<0.05 compared to Week 0 score; *** p<0.001 compared to Week 0 score.

The improvements at each post-treatment assessment were compared between the two groups. At Week 1, on average, the Test Group had 4.83 more improvement than the Control Group (95% C.I.=[1.30, 8.37], p=0.01). In terms of the percent improvement from the baseline, the Test Group had 26.6% improvement compared with 1.03% in the Control Group. At Week 2, the mean improvement in the Test Group was 5.63 greater than that in the Control Group (95% C.I.=[0.39, 10.96], p=0.03), and the percent improvement from the baseline in the Test Group was 39.2% vs. 10.6% in the Control Group. The mean improvements at Weeks 3 and 4, respectively, were still greater in the Test Group, but were not statistically significant. At Week 3, the mean improvement in the Test Group was 4.06 greater than that in the Control Group (95% C.I.=[-1.23, 9.36], p=0.21), with the percent improvement 48.2% Test Group vs. 30.2% Control Group. At Week 4, the Test Group on average had 2.83 more improvement than the Control Group (95% C.I.=[-4.39, 10.06], p=0.59), with the percent improvement 45.2% Test Group vs. 34.2% Control Group.

CBPI Pain Inference Score

The mean CBPI pain interference scores within each treatment group assessed at Week 0 and at Week 1 to 4 after the treatment started were calculated (Figure 3 and Table 5). The Week 0 CBPI pain interference score in the Control Group was 34.50±17.60 and was 33.75±11.02 in the Test Group. The difference was not statistically significant (p=0.71), which suggests group comparability on the pre-treatment pain interference condition.

At Week 1, the Control Group's CBPI pain interference score had a clear reduction to 26.42±13.22 (improvement=8.08±12.30, p=0.099), and further reduced to 25.25±18.20 (improvement=9.25±12.89, p=0.025) in Week 2, 16.50±13.45 (improvement=18.00±15.67, p=0.001) in Week 3, but was slightly increased to 17.00±16.57 (improvement=17.50±17.55, p=0.003) in Week 4. Statistically, significant improvement compared to the Week 0 score could be concluded from Week 2 and remained at Weeks 3 and 4.

For the Test Group, at Week 1, the CBPI pain interference score also greatly reduced to 24.25±14.08 (improvement=9.50±10.54, p=0.002), and further reduced to 21.12±14.10 (improvement=12.62±10.76, p=0.0004) in Week 2, 13.81±11.47 (improvement=19.94±9.41, p<0.0001) in Week 3, but also had an increase to 15.69±13.47 (improvement=18.06±11.47, p=0.0002) in Week 4. This suggests that similar to the observations in pain severity score, significant improvement on pain interference score was achieved after 1 week of treatment, and the improvement remained significant throughout the 4-week trial.

The treatment groups were compared for improvement of the pain interference score. The Test Group had a greater percent improvement than the Control Group at each post-treatment assessment: Test vs. Control were 28.2% vs. 23.4% (Week 1), 37.4% vs. 26.8% (Week 2), 59.1% vs. 52.2% (Week 3), and 53.5% vs. 50.7% (Week 4). The differences were smaller than those observed for pain severity score improvements. The overall group difference was not statistically significant, nor was each post-treatment assessment.

Table 5: Summary statistics (mean±SD) of CBPI pain interference score data

	Week 0	Week 1	Week 2	Week 3	Week 4
Control	Interference Score	Interference Score	Interference Score	Interference Score	Interference Score
	34.50±17.60	26.42±13.22	25.25±18.20	16.50±13.45	17.00±16.57
	Improvement from Week 0				
	—	8.08±12.30 (p_1=0.099)	9.25±12.89 (p_1=0.025)*	18.00±15.67 (p_1=0.001)**	17.50±17.55 (p_1=0.003)**
% improvement compared to baseline at start of study		23.4% improvement	26.8% improvement	52.2% improvement	50.7% improvement
Test	Interference Score	Interference Score	Interference Score	Interference Score	Interference Score
	33.75±11.02	24.25±14.08	21.12±14.10	13.81±11.47	15.69±13.47
	Improvement from Week 0				
	—	9.50±10.54 (p_1=0.002)	12.62±10.76 (p_1=0.0004)	19.94±9.41 (p_1<0.0001)	18.06±11.47 (p_1=0.0002)
% improvement compared to baseline at start of study		28.2% improvement	37.4% improvement	59.1% improvement	53.5% improvement
Group Comparison		p_2=0.590	p_2=0.274	p_2=0.390	p_2=0.639

p_1=p-value of improvement compared to Week 0; p_2=p-value of improvement comparison between groups; * p<0.05; ** p<0.01, *** p<0.001

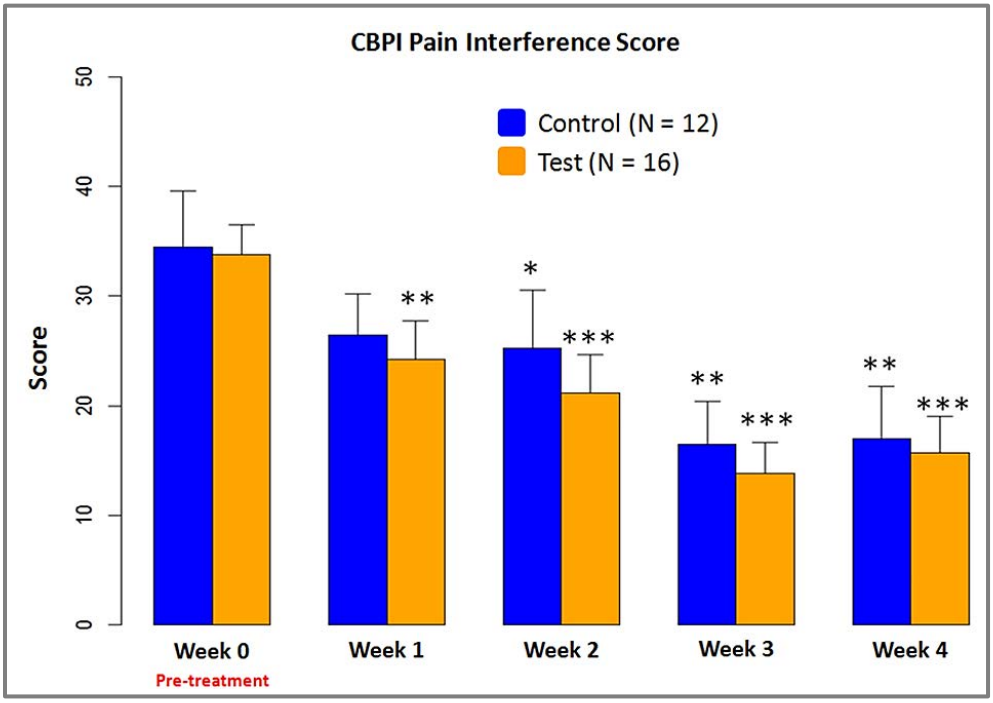

Figure 3: Mean (with standard error) CBPI pain interference score within each treatment group over time (pre-treatment Week 0 and 4 weekly post-treatment assessments). * p<0.05 compared to Week 0 score; ** p<0.01 compared to Week 0 score; *** p<0.001 compared to Week 0 score.

Quality of Life Score

The QoL score (an ordinal variable from 1 to 5) was also assessed and its improvement after receiving the treatment was compared between groups (Figure 4, Table 6). In the Control Group, the QoL score at Week 0 was 2.67±0.78 and was 2.88±0.72 in the Test Group. The difference was not statistically significant (p=0.63), which suggests group comparability for pre-treatment QoL condition.

After the treatment, the QoL score in the Control Group was improved. At Week 1, the QoL score increased to 3.08±0.79 (improvement=0.42±0.79, p=0.188), and further improved to 3.25±0.87 (improvement=0.58±0.90, p=0.094) in Week 2, 3.75±0.45 (improvement=1.08±0.90, p=0.004) in Week 3, but was slightly reduced to 3.67±0.78 (improvement=1.00±0.85, p=0.004) in Week 4. Statistically, improvement compared to the Week 0 score was significant from Week 3 and remained significant at Week 4.

Table 6: Summary statistics (mean±SD) of QoL score data

	Week 0	Week 1	Week 2	Week 3	Week 4
Control	QoL Score	QoL Score	QoL Score	QoL Score	QoL Score
	2.67±0.78	3.08±0.79	3.25±0.87	3.75±0.45	3.67±0.78
	Improvement from Week 0				
	—	0.42±0.79 p_1=0.188	0.58±0.90 p_1=0.094	1.08±0.90 p_1=0.004**	1.00±0.85 p_1=0.004**
Test	QoL Score	QoL Score	QoL Score	QoL Score	QoL Score
	2.88±0.72	3.38±0.89	3.50±0.73	3.75±0.77	3.88±0.96
	Improvement from Week 0				
	—	0.50±0.82 p_1=0.055	0.62±0.72 p_1=0.011*	0.88±0.72 p_1=0.001**	1.00±0.89 p_1=0.001**
Group Comparison		p_2=0.854	p_2=0.815	p_2=0.632	p_2=1.000

p_1=p-value of improvement compared to Week 0; p_2=p-value of improvement comparison between groups; * p<0.05; ** p<0.01, *** p<0.001

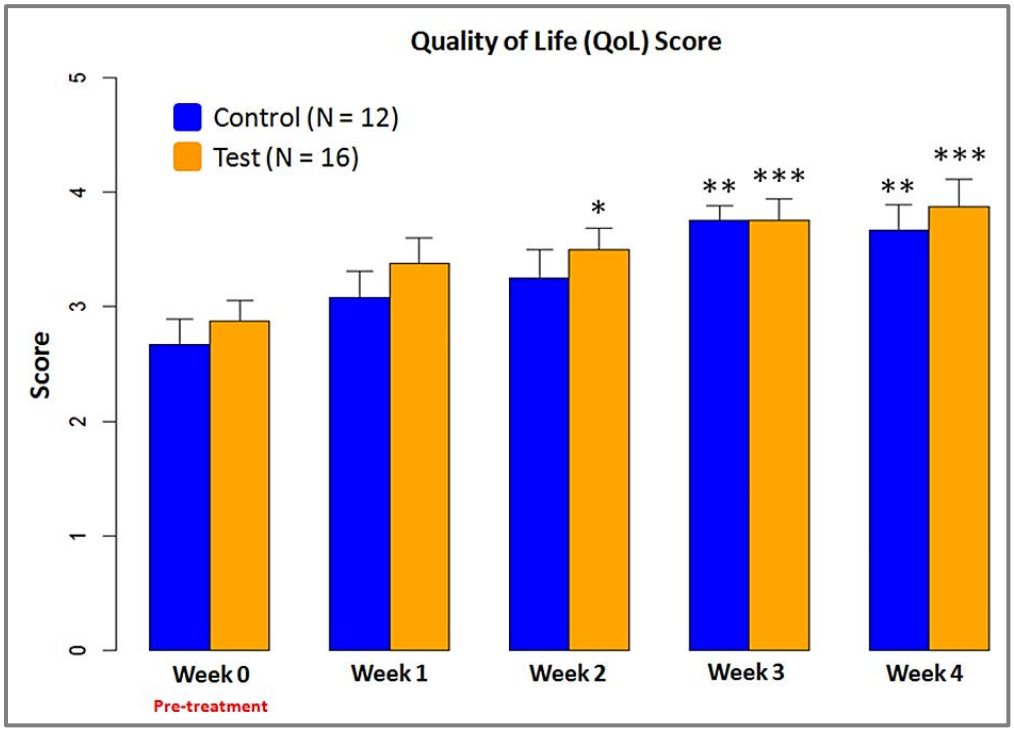

Figure 4: Mean (with standard error) QoL score within each treatment group over time (pre-treatment Week 0 and 4 weekly post-treatment assessments). * p<0.05 compared to Week 0 score; ** p<0.01 compared to Week 0 score; *** p<0.001 compared to Week 0 score.

For the Test Group, at Week 1, the mean±SD QoL score also improved to 3.38±0.89 (improvement=0.50±0.82, p=0.055), and further increased to 3.50±0.73 (improvement= 0.62±0.72, p=0.011) in Week 2, 3.75±0.77 (improvement= 0.88±0.72, p=0.001) in Week 3, and continued to increase to 3.88±0.96 (improvement=1.00±0.89, p=0.001) in Week 4. These observations suggested that, in the Test Group, significant improvement on QoL score was achieved after 2 weeks of treatment, and the improvement remained significant in Weeks 3 and 4.

DISCUSSION

The current study evaluated the efficacy of PSGAG[a] administration using aquapuncture injection technique compared with IM injection for treating canine OA patients. The data showed that, with either injection method, PSGAG treatment significantly improved the patient's pain condition (both severity and interference) and quality of life. The aquapuncture injection, however, demonstrated significantly more rapid and greater improvement during early treatment. The results from this

study suggest PSGAG administration using aqua-AP injection technique is non-inferior and potentially more effective and efficient than standard IM injection in mitigating pain severity for canine osteoarthritis patients.

Studies performed for the FDA new drug approval in 1997 demonstrated PSGAG[a] is moved to the joints from the intramuscular area quickly. Benefit is derived from the injectable due to its high sulfate content which allows it to bind to cartilage matrix through electrostatic interactions. By binding with the cartilage, PSGAG inhibits enzymes that cause injury to the cartilage tissue, which slows progression of structural changes to the joint, improves function and inhibits cartilage deterioration.[16,17]

The mechanism of action for PSGAG absorption and presence in joints following IM or aqua-AP administration is unknown; however, if absorption of PSGAG were the same for both groups in this study, the outcomes should be the same. Instead, findings from this study demonstrate there is a difference in treatment effects between these 2 techniques, suggesting the difference detected is likely due to the acupoint. Of primary consideration is the tissue differences at acupuncture points compared to non-acupuncture points (i.e. intramuscular). Acupoints are unique complex sites, usually located in fascia, which have a high density of arterioles/venules, nerve endings, lymphatics and mast cells. When stimulated they can create both local and distant neuromodulation as well as a cascade of paracrine signaling.[18] Additionally, the increased vascularity of these unique anatomic sites most likely yield improved absorption. A study that investigated the permeation and migration of labeled contrast material injected at acupoints demonstrated correspondence with fascial anatomy as well as respective organs of the acupoints.[18,19] Future studies investigating pharmacokinetic comparisons would be of interest.

Although the study did not recruit the goal number of patients, it was considered successful with respect to owner's compliance, treatment procedure, data collection processes, and study outcomes. It is believed that other clinicians who have similar knowledge and skills in veterinary acupuncture would be able to duplicate the study and obtain comparable outcomes. The study protocol was developed so that both the control and test groups could benefit from enrollment, given the expected efficacy of injectable PSGAG for this patient population. The decision to use the same aqua-AP treatment frequency as in a standard PSGAG protocol was to avoid an additional variable (treatment frequency). Selection of acupoints were based on benefits for osteoarthritis and to provide balance in forelimb and hindlimb treatment. Frequency of treatment and acupoints used for typical acupuncture clinical treatments can vary greatly depending on the severity and type of condition being treated; therefore, point selection and frequency might not be optimal and would be an interesting area for further investigation. The question was whether the proposed aqua-AP injection method could enhance the medicine's efficiency and/or efficacy. As expected, pain, interference, and QoL scores were all shown to improve over the length of the study for both groups. The faster significant improvements for aquapuncture technique (i.e. Test Group) supported the study hypothesis.

The most challenging aspects to conduct of this study were the low enrollment numbers and complications due to unexpected variation among subjects. Two patients were admitted to the protocol on recommendation from veterinarians within the practice. The evaluation of records indicated both qualified for inclusion. When the baseline CBPI valuations were reviewed after the start of the protocol (and after the submission of the end of Week 1 evaluations), these 2 patients had very low pain scores and high QoL evaluations for their baseline data. This left little room for improvement. This problem could have been minimized if the initial baseline evaluations were scrutinized (for example, having two independent evaluations) before patients were included in the study. Other variations included 3 other dogs (1 control, 2 test dogs), all very arthritic geriatric large breed patients, which showed poor response to treatments. The main objective of the study was to use an injectable known for its efficacy and only compare the difference between the two injection methods; subjects that did not respond to the medicine would not be meaningful inclusions. Finally, the low study recruitment led to a statistically underpowered study which increased the possibility of having type II statistical error (false negative). This led to difficulty concluding statistical significance in outcome data.

For assessment of treatment success in each group, the CPBI system was chosen due to its reliability and value in arthritis studies. Range of motion, which is commonly used for OA treatment outcome measurement, was not used for this study due to the diverse nature of the affected joints in this protocol. Grading lameness by an independent blinded individual, using a canine OA staging tool such as COAST, would have been helpful but was also not feasible for this study due to the number of visits required and the difficulty coordinating additional visits with the available individual during the restrictions imposed by the COVID pandemic.

Other study limitations included inconsistent owner completion of the CBPI forms (although it was asked), varied OA disease (severity and number of joints affected), lack of radiographic inclusion criteria to confirm OA diagnosis, and potential variability of other factors not reported by owners during the study period (medications, supplements, diet, body weight, exercise). Ideally dogs that all had the same affected joint rather than different joints would have made this a more robust study. The original protocol planned to only include subjects with a radiographic change; however, most dogs available for enrollment did not have radiographic data available; therefore, this was removed from inclusion criteria. This additional data would have confirmed OA and provided additional baseline information for post-hoc investigation on treatment outcomes for each group. The lameness score, other than Grade 2 or greater for study inclusion was not recorded. Better tracking of the initial degree of lameness could have provided opportunity for post hoc analysis.

In conclusion, this randomized controlled canine clinical study demonstrated that both standard IM and the proposed aqua-AP injection of PSGAG[a] can significantly reduce OA pain severity and function interference, along with improving QoL. The aqua-AP injection, however, provided more rapid and greater improvement during the first 2 weeks of treatment. Further studies to evaluate optimal acupoints and frequency of PSGAG injections to treat canine OA are natural extensions of the present research, along with evaluation of other injectables using this modified acupuncture technique. Future investigations evaluating this treatment in combination with other therapies would also benefit clinicians folding this treatment into a multimodality OA protocol. The findings of this study offer clinicians early evidence-based proof of the efficacy of injecting PSGAG in acupuncture points using aquapuncture technique and should be a consideration for providing more rapid and effective pain reduction in OA affected dogs.

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FOOTNOTES

[a] Adequan, Regent Pharmaceuticals, Shirley, New York, USA

[b] R version 4.4.1. The R Foundation for Statistical Computing, Vienna Austria; http://www.R-project.org

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