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A pilot study of 4CYTE™ Epiitalis® Forte, a novel Nutraceutical, in the management of naturally occurring osteoarthritis in the dog.

Epiitalis in the osteoarthritic dog

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Abstract

The primary goal of this pilot study was to assess, the efficacy of a new nutraceutical, 4CYTE™ Epiitalis® Forte containing, as a standalone, a proprietary plant oil extract, Epiitalis®, in dogs presenting with signs of osteoarthritis (OA).

Fifty dogs aged 9.2 (± 3.2) years with signs of naturally occurring OA were included in this report. They were free of other co-morbidities and were not on any medications except for those utilized for managing their OA. In those dogs, the current treatments were kept to avoid any sudden changes in their disease management. The effects of the 4CYTE™ Epiitalis® Forte were assessed both at the beginning and at the end of a one-month long treatment period. The evaluation consisted of an objective lameness assessment (TPI% - total pressure index) using a gait analysis (GAITRite® Portable Walkway System), and a subjective quality of life questionnaire (QOL), the Helsinki Chronic Pain Index (HCPI). Additional exploratory objective measurements included the Symmetry Index (SI) and the fore/hind limb ratio (T/P TPI%).

Seventy-four percent (34/46) of dogs registered a numerical improvement in TPI% in their worse limb. Additionally, of the 93.5% of the dogs that improved their HCPI scores by at least 5% on the QOL, 79% demonstrated improvements in gait based on TPI%. Finally, there were improvements measured in both exploratory objective endpoints, SI and T/P TPI%.

These encouraging results will be used to develop a protocol for a follow-up placebo-controlled randomised study to confirm the efficacy of this new nutraceutical for dogs suffering from OA.

Keywords: Osteoarthritis, dogs, nutraceutical, gait analysis, quality of life

Introduction

Osteoarthritis (OA) is a slowly progressive and dynamic disease. Data from a variety of sources suggest that approximately 20% of dogs above the age of 1 year suffer from OA¹. The signs associated with the disease include lameness, stiffness and functional disabilities that are believed to result from joint pain and restricted movement of the affected joint(s). Initially, the pain is believed to be primarily due to local joint damage and inflammation; however, as the disease evolves, the constant joint remodelling and painful stimulation cause maladaptive nociceptive central pain processing in the spinal cord and brain, leading to a shift to neuropathic pain^{2,3}.

There is no specific treatment for OA, and its management is usually multimodal, including both pharmacologic and non-pharmacological approaches⁴. Epiitalis® is a proprietary oil extract from the plant *Biota orientalis* and is one of four ingredients in a product called 4CYTE™ Canine used orally to relieve pain in dogs suffering from OA. 4CYTE™ Canine is registered by the Australian Pesticides and Veterinary Medicines Authority (APVMA). This APVMA approved product contains four ingredients; 3 marine-derived, plus Epiitalis®. 4CYTE™ Epiitalis Forte contains only Epiitalis® and is formulated as an oral gel. The efficacy of 4CYTE™ Canine on reducing arthritic symptoms in dogs over a 28 days period has been previously reported⁵.

The anti-inflammatory and chondroprotective effects of an equine formulation of 4CYTE™ Canine (4CYTE™ Equine SEQ - Interpath Pty Ltd) on articular cartilage have been investigated both in vitro and in vivo⁶⁻⁸. These studies report significant inhibitory effects of a simulated digest of 4CYTE™ Equine SEQ™ and its constituents on interleukin-1 (IL-1) induced prostaglandin E2 (PGE2), and nitric oxide (NO) production by cartilage explants⁶. In addition, chondrocyte viability in the cartilage explants was enhanced by treatment with the simulated digest of either 4CYTE™ Equine SEQ™ or the *Biota orientalis* oil (i.e. Epiitalis®) alone⁶. Decreases in synovial fluid biochemical markers consistent with the in vitro findings were also observed in vivo in horses dosed with (4CYTE™ Equine SEQ™ and then challenged with low-dose intra-articular injections of IL-1, and in horses dosed with (4CYTE™ Equine SEQ™ beginning at the time of surgical removal of an osteochondral fragment^{7,8}. Those anti-inflammatory and cartilage protective effects highlighted the potential benefit of the product in the prevention and also the management of joint inflammation, in that species. Recently, the manufacturer has made a new oral gel formulation for dogs, 4CYTE™ Epiitalis® Forte which contains Epiitalis® as the only active

ingredient. The primary goal of this pilot study was to assess the efficacy of Epiitalis® as a stand-alone active in the orally-dosed gel formulation against both objective lameness and subjective quality of life endpoints. A further goal of this work was to collect sufficient data to enable the design and powering of a follow-up placebo-controlled randomised study.

Material and methods

Ethics approval for the study was provided via the University of Melbourne Animal Ethics Committee approval (ID number: 1914862.1). The study population was drawn from dog owners who provided consent and who were seeking treatment for their dog's joint disorders at the University of Melbourne Veterinary Hospital. Dogs were first screened for study inclusion based on a clinical history of OA and owner input. A final determination of a diagnosis of OA was made after physical examination and determination of degenerative joint disease by a veterinarian experienced in canine OA (TB). Any dogs with co-morbidities, or that were receiving treatments other than for managing OA were excluded from the study. Dogs included in the study were kept on their current medications (pharmaceutical or otherwise) to both avoid a sudden change in their disease management and to understand whether 4CYTE™ Epiitalis® Forte would add efficacy to existing OA treatment regimens.

4CYTE™ Epiitalis® Forte was given by the owners once a day at a dose determined by the manufacturer (40µL/10kg). On Day 28, owners were asked to comment on the ease of agent administration, as well as to confirm the volume given per day

The dogs enrolled in the study were assessed on a GAITRite® Portable Walkway System (<http://www.gaitrite.com>), for gait analysis. GAITRite is a portable walkway validated for use in dogs⁹. It records temporospatial variables, including TPI for each leg¹⁰. Sequential force plate assessments of each individual leg were made during each run on the portable walkway. As described previously, this multivariate approach maximises the amount of data captured in each walkway run while reducing the variability of the data^{10, 11}. This system has been used successfully in different clinically-based lameness studies in dogs^{12, 13}. In studies in heterogeneously sized groups of normal dogs, it has been demonstrated that TPI% (Total Pressure Index) is not significantly affected by dog size for data collected either at the walk or trot^{14, 15}. TPI% is functionally equivalent to %BDW (Body Weight

Distribution) and, since this variable is not influenced by stride frequency, it was identified to be the most accurate and useful measurement for clinicians when evaluating a heterogeneous group of dogs¹⁴.

In our work, each measurement was the mean of three successful recordings. TPI% provided by the GAITRite® is determined similarly to the percentage of body weight distribution (%BWD): the sum of peak pressure values recorded from each activated sensor by a paw during walkway contact/total sum of peak pressure values for all feet x 100¹⁶. The value for TPI% is expected to be 30 and 20 for each forepaw and each hind paw, respectively¹⁵⁻¹⁷. TPI% provided by the GAITRite® software was used to identify and assess the worst leg on day 0 of the study. This same leg was then assessed on study day 28 and the change utilized as the primary study outcome (i.e. change from Day 0 to Day 28). Improvement in the TPI% scores was defined arbitrarily as a numerical increase in the TPI% value of the worse limb^{18,19}.

TPI% was also used to calculate the Symmetry Index (SI) and the fore/hind limb ratio (T/P TPI%) as described previously^{10,17}.

On both days 0 and 28, each owner also filled out the Helsinki Chronic Pain Index (HCPI)²⁰, a validated Quality of Life (QoL) questionnaire. Clinical improvement in the quality of life was defined arbitrarily as a decrease of 5% or more in the HCPI scores.

A Shapiro-Wilk test checked the normality of the data. Normally distributed data were compared using a paired T-Test; otherwise, a Mann-Whitney test was utilised. Differences were considered significant at $p < 0.05$. Unless otherwise indicated, all data are expressed as mean $\pm$ standard deviation; min-max).

Results

Of the fifty dogs enrolled in the study between May and September 2019, four dogs were eliminated from the final analysis. One dog due to a diagnosis of osteosarcoma, while in 3 dogs we were not able to obtain a consistent walkway reading on the second visit (Day 28) as the patients were too excited and unable to walk in a straight line. Forty-six dogs were therefore included in the study. The mean age and weight of the dog population were respectively 9.4 years (± 2.9 ; 3–16) and 27kg (± 18.5 ; 3.9–92). Results for the 46 dogs remaining are summarised in Tables 1 and 2

In terms of the primary outcome, 34 out of 46 dogs (74%) registered a numerical improvement in TPI% in their worse limb between days 0 and 28 (Table 1). For the secondary outcome, 43 out of 46 dogs (93.5%) improved their HCPI scores by at least 5% from Day 0 to Day 28 (Table 1). The mean improvement for the population was 26.6% (from 17.54 to 12.87; Table 2) and the change was highly significant ($p < 0.001$). Consistent with these data, 32 of the 34 dogs (94.1%) that exhibited an improved TPI% also recorded an improvement in their subjective HCPI score (Table 1).

As experimental outcomes, we also assessed two additional objective measurements, SI and T/P TPI%. In healthy animals, values of objective measurements obtained from the right and left forelimbs or between the right and left hind limbs should be similar, yielding an SI near 0, (perfect symmetry)^{10,17}. In our study, left to right SI went from -0.436 to -0.36, a 17% improvement (Table 2). When separating the dogs in 2 subgroups, those putting more weight on the left side ($n=25$) and those putting more on the right side ($n=21$), a significant difference ($p=0.002$ and 0.004 , respectively) before and after treatment was observed (Table 2). Overall, 26 dogs exhibited an improved SI, 9 did not improve while 11 showed a directional change in the SI.

When assessing the T/P TPI% ratio (front vs back) for the whole group, the treatment with 4CYTE™ Epiitalis® Forte resulted in a slight positive change from a ratio of 1.33 to a ratio of 1.38 (Table 2). Separating the dogs based on a front vs back lameness, two unequally represented groups emerged, with 34 dogs with front limb lameness versus 12 dogs with a hind limb lameness. Of those dogs, looking at the TPI% values at the start and at the end of the study, 24/34 (73%) and 9/12 (75%) of the dogs improved (Table 2).

Twenty-one dogs were still receiving OA related drugs when starting the study. Fifteen of those dogs (15/21; 71%) belonged to the group that had positive results on both the walkway and the questionnaire (Table 1). Four (4) dogs (19%) were from the group that did not improve on the walkway while improving on the questionnaire. One dog was from the group that improved on the walkway but not with the questionnaire. The last dog was from the group that did not improve anywhere. There were no statistical differences in terms of improvement between the dogs with and the dogs without concurrent OA related medications.

Concerning safety, the owners of two dogs reported softer faeces at the beginning of the 4CYTE™ Epiitalis® Forte treatment. In both cases, this resolved by itself. No other reports of possible side effects associated with the intervention were reported.

Discussion

In terms of the efficacy of 4CYTE™ Epiitalis® Forte, the primary outcome of this study was the effects of the intervention on the objective lameness measurement (TPI%). In our study, 34 out of the 46 dogs treated with 4CYTE™ Epiitalis® Forte registered an improvement between days 0 and 28 in their most affected limb. Consistent with the improvements in TPI%, additional objective measurements related to lameness (SI and T/P TPI%) also demonstrated improvements that favoured the 4CYTE™ Epiitalis® Forte intervention. In the secondary outcome (HCPI), 43 out of the 46 dogs treated with 4CYTE™ Epiitalis® Forte (93.5%) improved their HCPI scores by at least 5% from Day 0 to Day 28, with a mean improvement for the population of 26.4%. The subjective survey utilised in this study as the secondary outcome is the validated HCPI score²¹. Although other pain questionnaires could have been used, this one has the advantage that it can be completed by either the dog owner or their veterinarian. In addition, it correlates well with different chronic pain questionnaires such as the Canine Brief Pain Inventory (CBPI) and the 'Liverpool Osteoarthritis in Dogs' (LOAD) questionnaire²⁰.

HCPI is a subjective score, and a caregivers' placebo effect of up to 57% has been reported in a previous OA study²². Despite this, in our study, the data from subjective and objective measurements were remarkably consistent as, amongst the dogs with a positive subjective HCPI outcome, 67 % of them also improved on the hard objective primary outcome (TPI%).

The Symmetry Index has been used in different studies to characterise dogs of different sizes and to differentiate between lame and clinically healthy dogs^{10, 17, 23}. In a recent study, Kano et al. showed that mean SI values hovered around 0 and concluded SI could be used to evaluate the gait in a heterogeneous group of dogs¹⁴. In this study, while a majority of dogs (26) improved in their SI, 11 dogs showed a directional change. The leash side and the handler have been reported as a possible cause of side-shifting^{15, 24}. In the current study, while the same handlers walked the dogs on the walkway, we cannot exclude, (although asked not to), that some owners changed leash side, influencing our data. In dogs, as well as other species such as horses, dominant forelimb, behavioural lateralisation or paw preference have been reported^{15, 25-27}. Therefore, while getting better, some dogs

might have reverted to a more natural walk characterised by limb preference. Another explanation may include pain attenuation uncovering a mechanical lameness on an opposite limb. Finally, one cannot rule out a recent trauma on an opposite limb as a cause for side shifting.

It is accepted that about 60% of a dog's weight is carried by the forelimbs while the rest by the hindlimbs (fore/hind limb weight ratio of 1.5). Although it has been reported that there is some variation in this ratio between dogs from different breeds and with different stride lengths, 1.5 is still acceptable in a heterogeneous population such as ours¹⁴⁻¹⁷. In our study 34 out of 46 dogs demonstrated a change in T/P TPI% that favoured a move towards the theoretical value of 1.5, with 73% and 75% of the dogs with a front and a hind limb lameness, respectively, improving. The lack of significant difference for the hind limb subgroup probably resulted from a low sample size in this group.

Of interest, 15 out of 21 dogs that were previously on some kind of an intervention for OA, demonstrated improvements in both the objective primary outcome (TPI%) and the subjective secondary outcome (HCPI). Except for two dogs (intraarticular steroid injection in the hip four weeks earlier, and pentosan two months earlier), the prior medications had been started greater than three months previously. Although it is theoretically possible that over the period of the trial, there was some delayed benefit from prior/ongoing interventions, we think that this was unlikely due to the overall chronicity of the population's prior OA treatments. This supports the hypothesis that 4CYTE™ Epiitalis® Forte was able to provide efficacy that added to any benefit being provided by prior/ongoing OA-related interventions. At this time, we do not know the reason for this added benefit; however, we postulate that 4CYTE™ Epiitalis® Forte is working to either enhance the efficacy of pathways influenced by existing medications or may be working to provide benefit to pathways that are independent of existing medications.

In summary, treatment with 4CYTE™ Epiitalis® Forte significantly improved both objective lameness scores and subjective QoL scores in a population of dogs with pre-existing lameness due to joint OA. These results are encouraging and will be confirmed in a randomised placebo-controlled study in a similar population.

In summary, Epiitalis® as a stand-alone active in the orally-dosed gel formulation 4CYTE™ Epiitalis® Forte led to numerical improvements in the primary objective outcome (TPI%) in 34 out of 46 dogs

(74%) with clinical osteoarthritis. In addition, 43 out of the 46 treated dogs (93.5%) improved their subjective HCPI scores by at least 5% from Day 0 to Day 28. The very favourable data from this pilot study of 4CYTE™ Epiitalis® Forte will be used to refine study endpoints and power a follow-up placebo-controlled randomised study.

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Table 1. Summary of the dogs that either improved or did not improve vs. both the primary endpoint (TPI%) and vs. the secondary endpoint (HCPI), in a dog population (n=46) suffering from osteoarthritis and receiving 4CYTE™ Epiitalis® Forte.

Improvement vs. non-improvement was either (1) considered individually, or (2) considered as a combination of the two endpoints. The data vs. TPI% and HCPI were further considered within the subgroup that had been receiving prior stable medications for their OA

	Improved	Not Improved	Total
All dogs (46)			
TPI%	34	12	46
HCPI	43	3	46
Improved TPI% dogs (34)			
HCPI	32	2	34
SI	22	12	34
Improved HCPI dogs (43)			
TPI%	34	9	43
SI	26	17	43
Improved SI (26)			
TPI%	22	4	26
HCPI	26	0	26
Dogs with Medications (21)			
TPI% and HCPI	15	1	16
HCPI only	4	NA	5
TPI% only	1	NA	1
Dogs without medication (25)			
TPI% and HCPI	17	0	17
HCPI only	7	NA	7
TPI% only	1	NA	1

Table 2. Summary of efficacy data relating to the Helsinki Chronic Pain Index (HCPI), the Symmetry Index (SI) and the T/P TPI% (thoracic/pelvic limb weight ratio) in a group of dogs suffering from osteoarthritis and receiving 4CYTE™ Epiitalis® Forte.

The SI was assessed overall for each dog, but also as subgroups favouring either the left (-) or the right (+) side. The T/P TPI% was assessed overall for each dog, but also as subgroups showing either front or back limb-based lameness. A Shapiro-Wilk test checked the normality of the data. Normally distributed data were compared using a paired T-Test; otherwise, a Mann-Whitney test was utilised. Differences were considered significant at $p < 0.05$.

	Mean	St Dev	Min	Max	P values
HCPI scores					
Before	17.54	7.67	2	36	P< 0.0001
After	12.87	7.15	0	30	
SI all group					
Before	-0.436	2.3	-4.7	4.7	0.904
After	-0.36	2.0	-5.7	4.9	
SI left side					
Before	-2.15	1.3	-4.7	-0.1	0.002
After	0.83	2.1	-5.7	3.6	
SI rightside*					
Before	1.6	1.3	0.1	4.7	0.004
After	0.2	1.7	-2.6	4.9	
T/P TPI%					
Before	1.33	0.2	0.9	1.9	0.075
After	1.38	0.2	0.9	1.8	
T/P TPI% Front					
Before	1.24	0.1	0.9	1.5	0.025
After	1.31	0.2	0.9	1.7	
T/P TPI% Back*					
Before	1.60	0.1	1.4	1.9	0.862
After	1.59	0.1	1.2	1.8	

* data not normally distributed

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