Anti-inflammatory response of dietary vitamin E and its effects on pain and joint structures during early stages of surgically induced osteoarthritis in dogs

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Abstract

There is evidence that vitamin E (VE) has anti-inflammatory and analgesic properties in human osteoarthritis (OA). This double-blinded and randomized pilot study used a broad spectrum of clinical and laboratory parameters to investigate whether such beneficial effects could be detected in a canine experimental OA model. Dogs were divided into 2 groups: control (n = 8), which received a placebo, and test group (n = 7), which received 400 IU/animal per day of VE for 55 d, starting the day after transection of the cranial cruciate ligament. Lameness and pain were assessed using a visual analogue scale (VAS), numerical rating scale (NRS), and electrodermal activity (EDA) at day 0, day 28, and day 55. Cartilage and synovial inflammation lesions were assessed. One-side comparison was conducted at an alpha-threshold of 10%. At day 56, dogs were euthanized and concentrations of prostaglandin E_2 (PGE_2), nitrogen oxides (NO_x), and interleukin-1 beta (IL-1β) were measured in synovial fluid. Concentrations of NO_x and PGE_2 in synovial fluid were lower in the test group ($P < 0.0001$ and $P = 0.03$, respectively). Values of VAS, NRS, and EDA showed a consistent trend to be lower in the test group than in the control, while statistical significance was reached for VAS at day 55 and for EDA at day 28 (adjusted $P = 0.07$ in both cases). Histological analyses of cartilage showed a significant reduction in the scores of lesions in the test group. This is the first time that a study in dogs with OA using a supplement with a high dose of vitamin E showed a reduction in inflammation joint markers and histological expression, as well as a trend to improving signs of pain.

Résumé

La vitamine E (VE) est connue par ses propriétés anti-inflammatoires et analgésiques dans le traitement de l'ostéoarthrose (OA) chez l'humain. Dans notre étude pilote nous avons utilisé un ensemble de paramètres cliniques et de laboratoire afin de déterminer si ces effets bénéfiques de la VE pourront être détectés chez le chien arthrosique, dans un modèle expérimental d'OA. Les chiens utilisés ont été divisés en 2 groupes : témoin (n = 8), qui a reçu un placebo et un groupe supplémenté (n = 7), qui a reçu 400 UI de VE/animal/jour pendant 55 jours, la supplémentation orale a commencé un jour après la section du ligament croisé crânial. Avant la chirurgie (J0), J28 et J55 après chirurgie, la boiterie et la douleur ont été évaluées à l'aide d'une échelle visuelle analogique (EVA), d'une échelle d'évaluation numérique (NRS), et par la mesure de l'activité électrodermique (EDA). Les lésions au niveau du cartilage et l'inflammation synoviale ont été évalués. Une seule comparaison statistique a été réalisée avec un seuil alpha à 10 %. Au jour 56, les chiens ont été euthanasiés et les concentrations de prostaglandine E_2 (PGE_2), d'oxyde d'azote (NO_x) et d'interleukine-1 bêta (IL-1β) ont été mesurées dans le liquide synovial. Les concentrations synoviales de NO_x et de PGE_2 étaient plus faibles dans le groupe traité (P < 0,0001 et P = 0,03, respectivement). Les valeurs de l'EVA, de NRS et de l'EDA ont montré une tendance constante à être plus faible dans le groupe traité par comparaison au groupe témoin, avec un effet significatif de la VE qui a été observé pour VAS au jour 55 et EDA au jour 28 (P ajustée = 0,07 dans les deux cas). Les analyses histologiques du cartilage ont montré une réduction significative des scores lésionnels chez le groupe traité. Cette étude est la première à démontrer qu'une supplémentation orale avec une dose élevée de VE chez des chiens arthrosiques permet de réduire la libération des marqueurs inflammatoires et les lésions histologiques au niveau du cartilage, ainsi qu'une tendance à améliorer les signes de douleur.

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Introduction

Osteoarthritis (OA) in dogs is a slowly progressive, degenerative, and active disease. In terms of lesion changes, OA is characterized by degeneration of articular cartilage, with loss of matrix, fibrillation, and formation of fissures, which can result in a complete loss of the cartilage surface (1). In OA, synoviocytes and synovial macrophages produce a wide array of inflammatory mediators including prostaglandins (PGs), reactive oxygen species (ROS), and pro-inflammatory cytokines such as interleukin-1 beta (IL-1β), IL-6, and tumor necrosis factor alpha (TNF-α). In turn, these pro-inflammatory cytokines stimulate articular chondrocytes and synoviocytes to produce matrix-degrading enzymes such as matrix metalloproteinases (MMPs) and pro-inflammatory enzymes such as cyclooxygenase-2 (COX-2). The subsequent release of PGs promotes, sustains, and enhances additional cytokine production and inflammation, which leads to cartilage extracellular destruction and degeneration (2,3). Reactive oxygen and nitric oxide (NO) species inhibit collagen and proteoglycan synthesis, activate MMPs, increase the susceptibility of cartilage to injury by other oxidants, and induce chondrocytes apoptosis (4).

Vitamin E (VE) is the principal and most potent lipid-soluble antioxidant found in human erythrocytes (5). It also decreases the ability of monocytes to release ROSs such as hydrogen peroxide and superoxide anion and consequently reduces lipid oxidation of cell membranes (5). Vitamin E affects the synthesis of nitric oxide (NO) by inhibiting the activation of the transcription nuclear factor kappa B (NF-κB), which is implicated in NO synthase gene expression (6). Many studies have shown the anti-inflammatory effects of VE, with actions on several enzymes involved in inflammatory pathways, such as protein kinase C, protein phosphatase 2A, 5-, 12-, and 15-lipoxygenases, phospholipase A_2 (PLA_2), and COX-2 (7–9). In addition, VE supplementation induced suppression of a potentially atherogenic cytokine, IL-1β, and inhibition of a crucial event in atherogenesis, monocyte-endothelial cell adhesion (10). Consumption of VE has been associated with a lowered risk of coronary heart disease and reduced oxidation of low-density lipoprotein (11). Furthermore, VE plays a major role in regulating arachidonate release from membrane phospholipids and its subsequent metabolism to bioactive lipids such as PGs, prostacyclin, leukotrienes, thromboxanes, and other inflammatory mediators (12).

The antioxidant and anti-inflammatory properties of VE related to cardiovascular pathologies have been translated to the OA field. Several clinical human studies have found that VE has therapeutic benefits on OA symptoms over a short-term period. A double-blinded study showed an improvement in pain at rest and during movement after a 6-wk supplementation of 400 IU/d of α-tocopheryl acetate compared to a placebo as evaluated on a global assessment scale of pain (13). Another 3-week, double-blinded study demonstrated a similar efficacy of VE (544 mg of α-tocopheryl acetate 3 times a day) to diclofenac (50 mg 3 times a day) in decreasing pain of OA patients using a visual analogue scale (VAS) (14). In long-term, double-blinded studies, however, a daily dose of 500 IU of VE showed no symptomatic or structure-modifying benefits over a placebo, as assessed by Western Ontario and McMaster Universities Osteoarthritis Index or by magnetic resonance imaging (MRI) of OA in the knee (15,16).

In human patients with OA, vitamin E has been shown to improve clinical symptoms and reduce free radicals and synthesis of pro-inflammatory cytokines. In dogs with OA, VE could reduce clinical symptoms and synthesis of free radicals and slow articular cartilage lesions. The objective of the present study, therefore, was to assess the effect of a high dose of α-tocopherol supplementation on pain assessment, inflammatory markers, and structural changes in the joints of dogs with OA in experimental cranial cruciate ligament (CCL) transection model.

Materials and methods

Dog selection

Fifteen adult crossbred dogs, 1 to 4 y old, weighing 23.6 to 30.5 kg, were used in this study. The dogs were housed in a large kennel in individual galvanized steel cages [1 m (width) × 1.75 m (length) × 2.4 m (height)]. All cages were equipped with an automatic watering system. Dogs were selected after complete physical and musculoskeletal evaluations by a veterinarian. The dogs were subjected to hematological and biochemical analyses, the results of which had to be within normal range before being included in the study (17). Animal care procedures followed the guidelines of the Canadian Council on Animal Care and the protocol was approved by the Institutional Animal Care and Use Committee (10-Rech-1463).

Anesthesia and surgical procedures

Seven days before surgery, baseline pain and functional outcome levels were evaluated. All anesthetized dogs were subjected to a surgical sectioning of the CCL of the right knee as described in a previous study (17). A fentanyl patch (Duragesic; 50 or 75 μg/h, Janssen Ortho, Markham, Ontario) was placed in the dogs' necks 24 h before surgery. Before surgery, they received a subcutaneous analgesic premedication (meperidine, acepromazine, and glycopyrrolate), followed by intravenous induction of anesthesia with propofol. Dogs were endotracheally intubated and maintained on a 2% isoflurane/oxygen anesthetic mixture. A medial arthrotomy was done, distal to the patella and parallel to the patellar ligament. A retractor was inserted to view the CCL to be sectioned, the completeness of which was verified by obtaining a large drawer motion in both flexion and extension. The capsule and the retinaculum were sutured in a simple continuous pattern. Bupivacaine (Marcaïne 0.5%; Hospira, St. Laurent, Quebec) was injected (5 to 8 mL) in the capsule as an intra-articular block. Finally, the subcutaneous tissues were sutured, followed by intra-dermal and skin sutures.

Post-operative and oral supplementation procedures

Dogs were exposed to a progressive resumption of physical activity from day 2 to day 6 after surgery. After the first week, all dogs were actively exercised in exterior runs [1.35 m (width) × 9.15 m (length)] at the same facility for a 2-h period, 5 d a week, under the supervision of an animal care technician (18).

All dogs were fed the same food (Teklab Global 27% Protein Dog Diet; Harlan, Montreal, Quebec) starting 2 mo before surgery until the end of the experiment. Food quantities were adjusted

according to animal weight. Dogs were randomly separated into 2 experimental OA groups: a control group (n = 8) consisted of OA dogs that received an oral placebo solution (polyethylene glycol 400 monooleate, polysorbate 80, and n-propyl alcohol) and a test group (n = 7) consisted of OA dogs given VE orally, once a day, as liquid α-tocopheryl acetate, 0.044 mL/kg bodyweight (BW) (Rovimix E-40% 400 IU/mL; DSM Nutritional Products, Fort Worth, Texas, USA). This dosage (~400 IU per dog once a day) is 10 times the daily dosage of VE recommended by the Association of American Feed Control Officials (19). It is far from a toxic dose, however, as dogs tolerate high levels of VE (1000 to 2000 IU/kg of food) without adverse effects (19,20). Treatment was initiated the day after surgery and continued until day 55.

Collection and analysis of blood samples

At days 0, 21, 42, and 55 post-surgery, sera were obtained after blood centrifugation for 15 min at 2000 × g to measure VE. Vitamin E was determined by high performance liquid chromatography with ultra-violet detector (HPLC-UV), using Hewlett Packard Series 1100 liquid chromatograph (Agilent Technologies, Mississauga, Ontario) according to the method published elsewhere (21) and modified in our laboratory. Briefly, samples were isocratically eluted with a mix mobile phase (75/25) respectively of acetonitrile and methanol (Fisher Scientific, Nepean, Ontario) and detected at 285 nm wavelengths through a Hewlett-Packard 1046 fluorescence diode-array detector (Agilent Technologies). Column type was Zorbax Eclipse plus C18, 3.0 x 50 mm, 600 bars (Agilent Technologies, Santa Clara, California, USA). External and internal standards were prepared from α-tocopherol and α-tocopherol acetate (Sigma-Aldrich Canada, Oakville, Ontario) and were injected before samples were taken. Concentrations of VE were determined in microgram/deciliter (μg/dL) from a standard curve of the peak-area ratio of the analyte-internal standard, plotted against the concentration of analyte. Conditions of compliance criteria and quality control included: coefficient of determination of standard curve $(r) > 0.98$; relative standard deviation (rsd) ≤ 20%; detection limit (LOD) = 85% to 115%, and quantification limit (LOQ) = 80% to 120%. This method was also used to determine the VE concentration in synovial fluid after the dogs were euthanized (day 56). Standard hematological and biochemistry analyses were carried out on day 0 and day 56 by the Diagnostic Service of the Faculty of Veterinary Medicine at the University of Montreal.

Inflammation assessment

After the dogs were euthanized (day 56), synovial fluid was collected from stifle joints using a standard arthrocentesis technique. As a control in the same animal, the unaffected left stifle joint was tried for synovial sampling. Because of the limited and inconsistent amounts of collected fluid, however, only fluid from the right stifle joints was analyzed. Synovial fluids were immediately centrifuged (14 000 × g, 15 mn at 4°C) and concentrations of PGE_2 were measured by an enzyme-linked immune sorbent assay (ELISA) kit from Cayman Chemical (Ann Arbor, Michigan, USA) with a quantification limit of 15 pg/mL (17). Measurements were done in duplicate and results were expressed in picograms in the total joint synovial fluid (17). Levels of IL-1β in synovial fluid were determined in duplicate using a commercial dog IL-1β ELISA Kit (Bethyl Laboratories, Montgomery, Texas, USA) (22). Results were expressed as picograms/milliliter (pg/mL) in total joint synovial fluid.

Nitrites and nitrates (NO_x) levels (nmol/knee) were measured using a Sievers NO Analyzer (280i; Sievers Instruments, Boulder, Colorado, USA). This assay is based on spectrophotometric analysis following a chemiluminescent reaction between NO and ozone (23). Each sample (0.025 mL) was placed into a purge vessel containing 5 mL of vanadium chloride (VCl_3) and the solution was heated to 95°C. Samples were analyzed in duplicate (values averaged) and plotted against a calibration curve obtained from known concentrations of nitrate solution as described in a previous study (23).

Pain assessment

Lameness or signs of pain were assessed subjectively on day 0, day 28, and day 55 after CCL transection. The same veterinary technician, blinded to experimental design, did all scoring throughout the experiment. To record any changes in the posture and behavior of dogs, the technician assessed each dog by using a VAS (0 mm, "no" to 100 mm, "worst imaginable" pain length) completed in rest (static) and in dynamic position (17). The composite numerical rating scale (NRS) includes the 7 following criteria (17): global assessment (score 0 to 4); evaluation of lameness while the dog is standing up (score 0 to 4), walking (score 0 to 4), and trotting (score 0 to 4); willingness to hold up contralateral limb (score 0 to 4); evaluation of response to palpation (score 0 to 4); and evaluation of response to flexion and extension (score 0 to 4).

Electrodermal activity (EDA) was measured to assess objective pain at day 0, day 28, and day 55 using a Pain Gauge system (PHIS, Dublin, Ohio, USA), which is a palm-sized electronic device that assigns a 'pain score' of 0.1 to 9.9 to indicate pain/stress levels, and following the manufacturer's instructions (24). The device was placed on the right palmar paw (ethanol-dipped and non-clipped) for 2 s for a measure in triplicate.

Structural assessments

Immediately after euthanasia, the right knees of the dogs were placed on ice and dissected to quantify gross morphological changes, including the presence of osteophyte formation and cartilage lesions. Two independent observers who were blinded to treatment group allocation graded the findings with a consensual value (17,18). Macroscopic lesion areas at the cartilage surface on the femoral condyles and tibial plateaus were measured (in mm^2) with an electronic digital calliper (Digimatic Caliper Model No. 2071M; Mitutoyo Corporation, Kawasaki, Japan). The depth of erosion was graded with scores ranging from 0 (a normal surface) to 4 (erosion extending to the subchondral bone). The degree of formation of osteophytes was graded by measuring the maximum width (mm) of the spurs on the medial and lateral femoral condyles (17,18).

Histological evaluation was done on sagittal sections of cartilage from the lesional areas of femoral condyle and tibial plateau as described in previous studies (17,18). Specimens were dissected, fixed in TissuFix #2 (Laboratory Gilles Chaput, Montreal, Quebec) for 24 h and embedded in paraffin. Serial sections (5 μm) were then stained with hematoxylin/fast green and Safranin-O. The severity of cartilage pathology was graded by 2 independent observers using

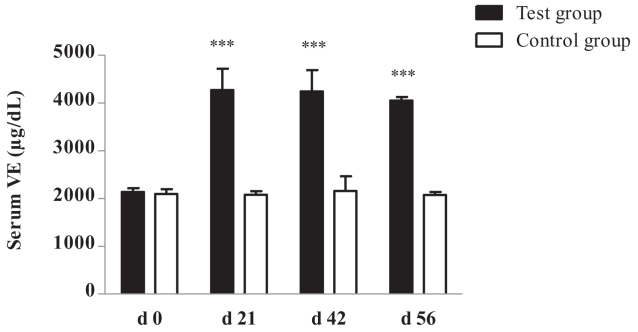

Figure 1. Concentrations of vitamin E in serum at baseline (day 0) and at day 21, day 42, and day 56 after beginning vitamin E supplementation. Each point corresponds to the mean (± SD) of 7 dogs for test group (solid bar) and 8 dogs for control group (open bar). * indicates a significant difference ($P < 0.001$).**

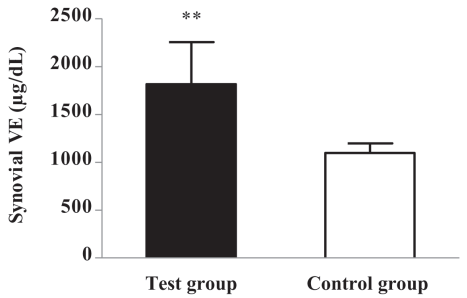

Figure 2. Concentrations of vitamin E in synovial fluid after euthanasia of dogs (day 56). Each point corresponds to the mean (± SD) of 7 dogs for test group (solid bar) and 8 dogs for control group (open bar). ** indicates a significant difference ($P < 0.01$).

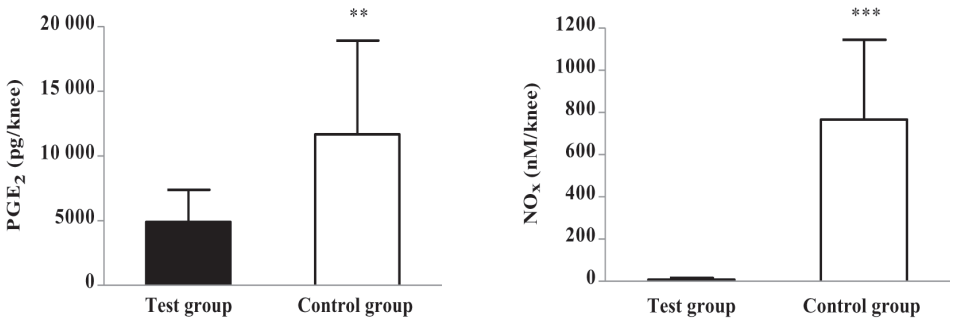

Figure 3. Amounts of prostaglandin E_2 (PGE_2) and nitrogen oxide (NO_x) in synovial fluid from osteoarthritic stifle joints. Each point corresponds to the mean (± SD) of 7 dogs for treated group (solid bar) and 8 dogs for control group (open bar). * indicates a significant difference ($P < 0.001$) and ** indicates a significant difference ($P < 0.01$).**

the Osteoarthritis Research Society International (OARSI) histopathology scoring system (25) in blind and consensual conditions. A scale of 0 to 29 was used to evaluate the severity of OA lesions based on the loss of Safranin-O staining (scale 0 to 4), cellular changes (scale 0 to 12), structural changes (scale 0 to 10), and pannus formation (scale 0 to 3). The final score (range 0 to 87) corresponds to the sum of the final scores for the 3 subregions of each specimen from the femoral condyle or tibial plateau.

Synovial membrane was removed and processed as described in a previous study (26). Samples were stained with hematoxylin-phloxine-saffron. The severity of synovitis was graded on a scale of 0 to 10 (17,18) by 2 blinded and independent observers. Histological criteria were synovial cell hyperplasia (scale 0 to 2), villous hyperplasia (scale 0 to 3), and mononuclear (scale 0 to 4) and polymorphonuclear (scale 0 to 1) cell infiltration; 0 indicates normal structure.

Statistical analysis

Statistical analyses were carried out using Student's *t*-test to compare biomarker inflammation levels in synovial fluid and lesion scores in the 2 groups of OA dogs after euthanasia. Repeated-measures analysis of variance (ANOVA) was used to compare pain and lameness assessments in the test and control groups. Values are expressed as mean ± standard deviation (SD). With regard to the preliminary exploration of the effects of VE on canine OA, sample size was limited (to $n \leq 8$ per group) for this study. We expected a between-groups difference of 20% in pain assessment methods with a sigma variation of 20% in the measurement. To maintain a desired beta power of analysis at around 80%, we therefore fixed *a priori* the alpha threshold of statistical significance for 1-sided test to the value of 10%. Sequential Bonferroni correction for multiple pairwise comparisons was applied when necessary.

Results

There was no significant change in the body weight of the dogs or evidence of any significant side effects of VE treatment during this study. Starting from the first time-point of analysis (day 21) to the end of the experiment ($P < 0.001$), concentrations of VE in serum were significantly higher in the test group than in the control group (Figure 1). The concentration of VE in synovial fluid was also significantly higher in the test group than in the control group ($P = 0.005$) (Figure 2).

Inflammatory joint markers

Amounts of PGE_2 and NO_x in synovial fluid were significantly lower in the test group than in the control group ($P = 0.03$ and $P < 0.0001$, respectively) (Figure 3), which indicates an effect of VE on inflammatory markers. In both groups, concentrations of IL-1β in synovial fluid samples were below the detection limit of the dog-specific ELISA kit used.

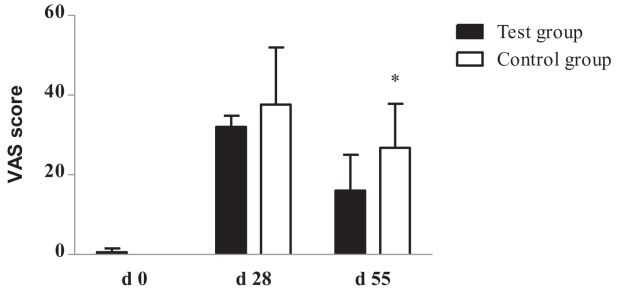

Figure 4. Changes in technician VAS assessment of force exerted by lame osteoarthritic dogs from baseline (day 0) to 28 d and 55 d after surgery. Each point corresponds to the mean (± SD) of 7 dogs for test group (solid bar) and 8 dogs for control group (open bar). * indicates a significant difference ($P < 0.1$).

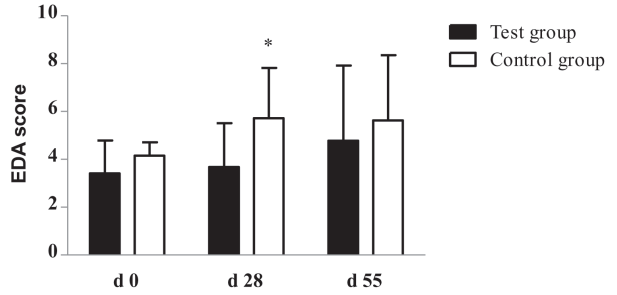

Figure 5. Changes in electrodermal activity (EDA) exerted by lame osteoarthritic dogs from baseline (day 0) to 28 d and 55 d after surgery. Each point corresponds to the mean (± SD) of 7 dogs for test group (solid bar) and 8 dogs for control group (open bar). * indicates a significant difference ($P < 0.1$).

Table I. Total histological scores for severity of articular cartilage lesions on femoral condyles and tibial plateaus in control and test dogs

	Total scores		
	Control group	Test group	*P*-value
Femoral condyles			
Lateral	11.30 (6.58)	5.48 (0.97)	0.04
Medial	12.21 (7.47)	7.67 (0.90)	0.13
Tibial plateaus			
Lateral	13.38 (5.52)	9.20 (1.57)	0.07
Medial	13.97 (5.97)	9.05 (2.37)	0.06

Score was determined as described in the materials and methods section. The total score corresponds to the sum of scores obtained for 3 subregions (0 to 87). Data are expressed as mean (± SD) and were analyzed using Student's *t*-test.

Lameness/pain assessment

Both VAS and NRS scores varied significantly (both $P < 0.0001$) within time, with a maximum reached at the intermediate time-point (day 28). At day 55, a difference was observed in the visual analogue score (VAS) taken by the veterinary technician between the test and control groups (adjusted $P = 0.07$) (Figure 4). The VAS pain score in the test group decreased by 40% at day 55 compared with the control group, this decrease was also present at day 28 as a trend (decrease of 15%). Similar evolution was observed for NRS, with a non-significant average decrease of 18% in the test group compared to the control group at day 55 (adjusted $P = 0.24$) and of 14% in favor of the test group at day 28 (adjusted $P = 0.36$).

Variation within-time of EDA was significant for the control group ($P = 0.009$), but EDA remained stable within-time for the test group ($P = 0.60$) (Figure 5). At day 28 and day 55, EDA values were, respectively, lower (adjusted $P = 0.07$) and not significantly different (adjusted $P = 0.27$) in the test group than in the control group.

Structural assessment

There were no differences in macroscopic grading, including osteophytes and cartilage lesions, in both groups of dogs (data not shown). Cartilage specimens from control dogs exhibited modifications that are typical of OA. The total histological scores for the severity of cartilage lesions on femoral condyles and tibial plateaus were decreased in VE-treated dogs (Table I). Vitamin E had a significant beneficial effect on lateral femoral condyle lesions ($P = 0.04$) and on both medial and lateral tibial plateaus ($P = 0.06$ and $P = 0.07$, respectively). This beneficial effect of VE was not observed, however, in medial femoral condyle lesions ($P = 0.13$). Moreover, there was no difference in histological lesion scores of synovial membrane between the 2 groups ($P > 0.1$).

Discussion

In the present study, concentrations of VE in serum and synovial fluid were significantly increased after dietary VE supplementation. Concentrations of VE found in serum of the control group (2098 ± 39 μg/dL) were similar to those previously reported for dogs. Thus, McLellan et al (27) found VE concentration in serum of 46.86 ± 16.5 μmol/L (2018 ± 710 μg/dL). The same results were found by Johnston et al (28), with a VE concentration in serum of 37.3 ± 13.2 μmol/L (1603 ± 567 μg/dL) in German shepherd dogs. In our study, supplementation with 400 IU of VE daily in the treated group generated a 2-fold increase of VE concentration in serum (4185.852 ± 118.97 μg/dL). Some studies conducted in dogs showed that the increased intake of VE was responsible for increased VE concentration in serum. Jewell et al (29) found an increase of 26% in VE concentration in serum in dogs when they doubled VE intake and an increase of 40% when VE intake was increased by 4-fold. In 2002, the same authors found an increase of 61% in VE concentration of serum when the dogs' diet was 3 times more concentrated in VE (30).

To our knowledge, the present study is the first to show that VE supplementation in early stages of surgically induced OA in dogs reduces the production of pro-inflammatory markers (PGE_2, NO_x) in synovial fluid. This vitamin also contributes to reducing histological lesions in articular cartilage and decreasing pain associated with the development of OA. Indeed, α-tocopherol appears to modulate a variety of cellular functions that are not necessarily a result of its antioxidant activity. In previous studies, Azzi et al (7) and Singh et al (9) showed that VE depresses PGE_2 biosynthesis, possibly by preventing the release of arachidonic acid and inhibiting PLA_2 and COX expression. This may explain results for PGE_2 in our study, which found that concentrations of PGE_2 in synovial fluid in the OA joints of dogs in the test group were lower than these concentrations in dogs in the control group.

In our study, NO_x concentrations decreased significantly in the OA joints of dogs in the VE-treated group compared to the control group. While normal cartilage explants produce little NO_x, chondrocytes and synovial membrane in OA and rheumatoid arthritis patients produce NO_x abundantly (31). This inorganic free radical, NO, promotes increased vasodilatation and vasopermeability associated with the inflammation of synovial tissue (32). The effect of NO_x on OA is likely to be exerted within the cartilage, where it promotes a number of catabolic effects on chondrocyte functions that would be expected to result in the loss of matrix, a feature of progressive OA (33). Vitamin E has effectively inhibited the activation of cytokine-induced NFκB, which plays a critical role in iNOS gene induction (34). Indirectly, VE acts as an antioxidant that inhibits IL-1β and TNF-α and oxidative stress, which in turn enhances NF-κB activation (35). In our study, VE supplementation led to a major decrease of synovial NO_x production.

Vitamin E apparently had no effect on concentrations of IL-1β in the synovial fluid. In previous human studies, VE therapy, especially at high doses, has been shown to decrease pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α in patients with cardiovascular disease. It has a biological effect of inhibiting the release of IL-1β by inhibiting the 5-lipoxygenase pathway (36). This discrepancy may be due to the fact that in our study IL-1β was measured 56 d after surgery. While this cytokine plays a role in initiating OA, there are other substances such as PGE_2 and MMPs that are produced to ensure the progression of OA. This probably explains the very low concentrations of IL-1β found in synovial fluid (37).

In our study, we found that, overall, subjective pain assessments (VAS and NRS) were in favor of an analgesic effect of VE. Our results are in agreement with other studies in human OA that show that VE was effective in decreasing short-term OA pain (13,14). Machtey and Ouaknine (38) also showed that in a small 10-d, single-blind crossover study on spondylosis, 600 mg of VE per day were better than a placebo as assessed by a patient questionnaire. The positive effect of VE on pain relief in OA demonstrated in short-term studies, however, has not been supported by the results of well-conducted studies over longer periods of time (15,16).

To objectively assess pain in dogs with OA, EDA was measured. The follow-up of EDA showed an improvement in pain (and stress) score in the VE-treated group compared to the control group at day 28. Most importantly, the level of EDA remained constant in the VE-treated group, whereas it increased as OA developed in the control group. Because EDA is very dependent on the dermal vasomotor tone, this could be related to the inhibition of inflammatory mediators as observed in our synovial fluid samples. It is recognized that PGE_2 is correlated with pain and functional disability in human OA (39). In the canine CCL model of OA, concentration of PGE_2 in synovial fluid is involved in acute inflammation and is associated with lameness and signs of pain during the early stage after CCL transection (40). It is likely that NO contributed to the disability and perception of pain (41). Vitamin E could exert its analgesic effect by interacting with NO (42). Our results support the finding that VE can improve short-term pain in dogs with OA.

In the present study, treatment with VE significantly reduced histological lesions in the articular cartilage in femoral condyles and tibial plateaus, but not in medial condyle lesions. Several studies have shown that VE has a positive effect on different compounds of articular cartilage. Tiku et al (43) showed that when rabbit chondrocytes were submitted to an oxidative burst, VE reduced collagen catabolism by preventing protein oxidation mediated by aldehydic down products of lipid peroxidation. Incubation of avian chondrocytes subjected to oxidative stress with VE restored collagen synthesis (44). These findings can explain the positive effect of VE in histological articular cartilage lesions in early stages of surgically induced OA in dogs in our study. Moreover, the decrease in NO_x levels after VE treatment in our study may have contributed to the protection of chondrocytes. It is surprising, however, that despite its effect in synthesis pathways of PGE_2 and NO_x, vitamin E could not reduce inflammation of the synovial membrane of dogs in the test group, even if this inflammation occurs early, i.e., 1 to 2 wk, after CCL section (45).

Our study has limitations that are primarily imposed by its design, including the duration of the study (8 wk) and the number of dogs used ($n \leq 8$). A study of longer duration would provide more information on the potential effects of VE on the long-term development of OA. Further investigation is required of the mechanisms of action of VE, especially its global effect on catabolic/anabolic factors, in order to better understand vitamin E's mechanism of action in OA pathways.

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