P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com
Research Article https://doi.org/10.47278/journal.ijvs/2021.107

Vitamin E as a Safe and Effective Vehicle for 1% Cyclosporine Eye Drops to Treat Chronic Non-Ulcerative Keratopathies in Dogs and Cats

Kevin Arteaga*, Emanuele Arnone and Manuela Crasta

Anicura Visionvet, Eye Clinic, San Giovanni in Persiceto, Bologna, Italy
***Corresponding author:** kevinof91@hotmail.com

Article History: 21-370 Received: 12-Aug-21 Revised: 03-Sep-21 Accepted: 10-Sep-21

ABSTRACT

To evaluate the efficacy of topical cyclosporine A (CsA) diluted in vitamin E (CsA-E) to treat keratoconjunctivitis sicca (KCS) and chronic superficial keratitis (CSK) in dogs and proliferative eosinophilic keratitis (PEK) in cats. In addition, we also aimed to assess the safety and tolerability of topical CsA-E for dogs and cats. A total of 146 client-owned animals with chronic keratopathies: 96 dogs with KCS, 27 with CSK, and 23 cats with PEK were included in the study. All animals underwent a complete ophthalmic examination at the Visionvet Eye Clinic (Bologna, Italy). Eligible animals received a drop of CsA-E from one to three times daily depending on the severity of the lesions and the response to treatment. We analyzed the evolution of several clinical treatment responses (CTR) parameters at baseline and at three and six-month follow-up. All CTR parameters improved significantly in KCS and CSK dogs and PEK cats after topical CsA-E treatment at three and six-month follow-up, except for those symptoms barely present in our sample at baseline. No systemic adverse events were detected, but two dogs and one cat showed conjunctival hyperemia and blepharospasm after topical CsA-E treatment. Topical CsA-E was established as an effective treatment for KCS and CSK in dogs and PEK in cats. Vitamin E was deemed safe and tolerable for its topical use in animals and could be considered an adequate alternative to other oil-based solvents when considering CsA ophthalmic preparations.

Key words: Vitamin E, Cyclosporine A, Keratoconjunctivitis sicca, Chronic superficial keratitis, Proliferative eosinophilic keratitis, Vehicle

INTRODUCTION

Cyclosporine A (CsA) is a lipophilic undecapeptide isolated from the fungus *Tolypocladium inflatum* with potent immunosuppressant activity (Gilger and Allen 1998). CsA has been shown to selectively inhibit T-lymphocyte activation and mast cell-mediated cytokine production and to completely inhibit IL-2, IL-3, IL-4, and granulocyte-macrophage colony-stimulating factor, hence rendering it an adequate treatment for allergic disease (Whitcup et al. 1996; Gilger and Allen 1998). Despite its effects on T-cell proliferation, CsA is not cytotoxic at therapeutic concentrations (Gilger and Allen 1998). In addition, CsA possesses interesting properties for its veterinary ophthalmic use. These include lacrimostimulant and lacrimomimetic effect, stimulant of mucin production (Moore et al. 2001) and reduction of corneal neovascularization and inflammatory cellular infiltrates (Kaswan et al. 1989; Kaswan 1994). Consequently, CsA has been extensively used to treat and improve clinical signs in some immune-mediated ocular diseases. In particular, CsA has shown to be effective in the treatment of keratoconjunctivitis sicca (KCS) (Kaswan and Salisbury 1990; Moore et al. 2001; Williams 1997; Dodi 2015) and chronic superficial keratitis (CSK) (Jackson et al. 1991; Williams et al. 1995) in dogs and in cats with proliferative eosinophilic keratitis (PEK) (Read et al. 1995; Spiess et al. 2009; Dean and Meunier 2013).

The lipophilic nature of CsA requires lipid solvents for its ophthalmic formulation. The most commonly used are olive, castor, sunflower, and corn oil, although artificial tears have also been reported as CsA solvent (Acheampong et al. 1998; Labbé et al. 2017). However, the bioavailability of CsA in these oils is low and they produce side effects in humans, including blurred vision, burning, and stinging and are, thus, poorly tolerated (de Oliveira and Wilson 2019). Therefore, new oil-in-water formulations are being investigated, such as gel systems, hydrogels, nanoparticles, liposomes, cationic emulsion, and penetration colloidal carriers (de Oliveira and Wilson 2019). Despite the promising results obtained with some of these novel drug delivery systems, their long-term safety and tolerability profiles have not yet been established (de Oliveira and Wilson 2019).

Cite This Article as: Arteaga K, Arnone E and Crasta M, 2022. Vitamin E as a safe and effective vehicle for 1% cyclosporine eye drops to treat chronic non-ulcerative keratopathies in dogs and cats. International Journal of Veterinary Science 11(2): 236-242. https://doi.org/10.47278/journal.ijvs/2021.107

Vitamin E is a family of molecules composed of four tocopherols and four tocotrienols (Pekmezci 2011; Peh et al. 2016). This essential nutrient possesses anticholesterolemic, anti-cancer, anti-inflammatory, cardioprotective, neuroprotective (Peh et al. 2016), and immunological properties (Pekmezci 2011). In addition, vitamin E is considered the most important antioxidant present in the body because of its activity against the damaging effects of reactive oxygen species (Mustacich et al. 2007; Pekmezci 2011; Peh et al. 2016; Miyazawa et al. 2019). These properties have fostered the use, in humans, of vitamin E to treat ocular diseases, such as age-related macular degeneration, cataract, uveitis (Xin et al. 2016), and to prevent keratocyte apoptosis after refractive surgery (Bilgihan et al. 2001). Besides, vitamin E has been shown to protect against UV radiation-induced cataract in albino rat animal models (Ayala and Söderberg 2004). Since PEK is an inflammatory disease (Dean and Meunier 2013) and KCS and CSK are both immune-mediated pathologies (in the case of CSK triggered by excessive UV exposure) (Nell et al. 2005), we hypothesised that the use of vitamin E as a vehicle for CsA would show positive results in these patients.

Our main objective was to evaluate the efficacy of topical CsA diluted in vitamin E (CsA-E) to treat KCS and CSK in dogs and PEK in cats. In addition, we also aimed to assess the safety and tolerability of topical vitamin E, used as a vehicle, for dogs and cats.

MATERIALS AND METHODS

Study Design

This was an uncontrolled interventional study using medical records from animals admitted to the Visionvet Eye Clinic (Bologna, Italy) between 2009 and 2021. The owners gave their written informed consent to use their data for our study. Medical records included date of presentation, breed, age, gender, eye affected, topical treatment, follow-up time, results of ophthalmic diagnostic tests, clinical treatment response (CTR) and complications. All animals underwent a complete ophthalmic examination by either a board-certified ophthalmologist or a supervised ophthalmology resident.

Participants

We included data from dogs diagnosed in our centre with KCS and CSK and cats with PEK. We diagnosed KCS based on the presence of characteristic clinical signs in conjunction with decreased quantitative tear readings (Schirmer tear test 1 [STT-1] <15 mm/min). In addition, we performed cytological evaluation in all CSK and PEK cases to confirm the clinical diagnosis. We excluded data from patients with a follow-up time of less than three months.

Materials

The ophthalmic examination included slit-lamp biomicroscopy (Keeler PSL Classic Portable Slit Lamp, Keeler Ltd, UK), STT-1, applanation tonometry (Tono-pen Vet, Reichert Inc, Depew, NY, USA), binocular indirect ophthalmoscopy (Vantage Plus Wireless, Keeler Instruments Inc., Broomall, PA, USA), and fluorescein ocular surface test. We prepared CsA-E in sterile conditions by diluting 1% CsA in 80% pure vitamin E (DL-α-tocopherol acetate, racemic mixture) and 20% medium-chain fatty acids from coconut oil.

Intervention

All eligible animals received a drop of CsA-E from one to three times daily, depending on the severity of the lesions and the response to treatment. Concurrent treatments included topical antibiotics, topical anti-inflammatories and/or ophthalmic lubricants based on disease response and clinician discretion.

Outcomes and Measures

We evaluated CTR by considering the following parameters: conjunctival hyperaemia, mucous discharge, corneal neovascularisation and blepharospasm. Additionally, we also assessed STT-1 values for KCS dogs, corneal pigmentation for KCS and CSK patients, and corneal infiltrates for CSK and PEK patients. Except for STT-1 values, we used a grading scale from 0 to 3 to classify CTR. The grading scale was as follows: Grade 0, not affected; Grade 1, mildly affected; Grade 2, moderately affected; and Grade 3, severely affected. We classified clinical signs for each parameter in our CTR according to this grading scale (Table 1). We analysed the evolution of these clinical parameters from baseline (T0) to follow-up at three months (T1) for all patients. We also analysed the follow-up at six months (T2), for which enough data was deemed enough for statistical comparisons.

Statistical Analysis

We described categorical variables as absolute frequencies and percentages and continuous variables as median and ranges. Data from the eye with a higher CTR in T0 was chosen for statistical analysis. We compared categorical variables between T0 and T1 using the non-parametric Wilcoxon test and between T0, T1 and T2 with the non-parametric Friedman test. We carried out all statistical analyses using SPSS software Version 24.0. We set the statistical significance level at $P<0.05$.

RESULTS

In our study, 146 animals were evaluated at T0 and T1, of which 96 had KCS, 27 had CSK and 23 had PEK. Data at T2 (six months of follow-up) from 91 animals were available, of which 60 with KCS, 14 with CSK, and 17 with PEK. The median follow-up was six months (range: 3-48 months).

Keratoconjunctivitis Sicca

The baseline characteristics of KCS dogs are summarised in Table 2. Most patients were intact male (41, 43%) and their median age was eight years (range: 0.3-14 years). Brachycephalic dogs accounted for 55% of the total KCS cases. All our KCS patients had STT-1 values below 15 mm/min at T0, whereas at T1 51% showed values above 15 mm/min ($P<0.001$) (Table 3). At T2, these proportions remained similar to those at T1 ($P<0.001$). For conjunctival hyperaemia, the number of patients presenting a grade 0 went from one (1%) to 64 (67%) between T0 and T1 ($P<0.001$), and, although 21 (22%) patients presented a grade 3 at T0, none were classified in this category at T1 ($P<0.001$). At T2, none showed a grade 2 conjunctival hyperaemia and only nine (15%) patients were classified as grade 1 ($P<0.001$). With varying proportions, the same trend as the one described for conjunctival hyperaemia was

Table 1: Grading scale of clinical signs to classify treatment response

	Grade 0	Grade 1	Grade 2	Grade 3
Conjunctival hyperaemia	Not affected	Mild	Moderate	Severe
Mucous discharge	Not affected	Small amount in the conjunctival sac	Discharge at medial canthus	Severe discharge across cornea or on eyelid margins
Corneal pigmentation	Not affected	< 25% of the cornea	25–50% of the cornea	>50% of the cornea
Corneal neovascularisation	Not affected	Limbal corneal vessels	Vessels halfway towards the corneal axis	Vessels extending to axial cornea
Blepharospasm	Not affected	Palpebral fissure slightly narrowed	Palpebral fissure 50% narrowed	Palpebral fissure completely closed
Corneal infiltrates	Not affected	< 25% of the cornea	25–50% of the cornea	>50% of the cornea

Table 2: Baseline characteristics of dogs and cats according to their pathology

	KCS[a] (n=96)	CSK[b] (n=27)	PEK[c] (n=23)
Sex, *n* (%)			
Female	27 (28)	1 (4)	–
Spayed female	21 (22)	16 (59)	4 (17)
Male	41 (43)	9 (33)	13 (57)
Castrated male	7 (7)	1 (4)	6 (26)
Age (years), *median* (range)	8 (0.3-14)	5 (3.0-12)	4 (1.0-16)
Follow-up time (months), *median* (range)	6 (3-36)	6 (3-48)	6 (3-48)

CSK: chronic superficial keratitis; KCS: keratoconjunctivitis sicca; PEK: proliferative eosinophilic keratitis: [a]Breeds, (n):English Cocker Spaniel (11), Shit-tzu (14), Mix (14), King Cavalier Charles Spaniel (9), English Bulldog (9), French Bulldog (5), Chihuahua (5), Maltese (5), Bolognese (3), West Highland White Terrier (4), Golden Retriever (1), Boston Terrier (1), Bull Terrier (1), Pug (5), American Hamstaff (1), Poodle (1), Jack Russel Terrier (1), Lhasa Apso (1), Pekingese (1), Yorkshire Terrier (4): [b]Breeds, (n): German shepherd (13), Mix (9), Cavalier King Charles Spaniel (1): [c]Breeds, (n): Domestic short hair (20), Chartreux (1), Ragdoll (1), Siberian (1).

Table 3: Evolution during follow-up of different clinical treatment response parameters in keratoconjunctivitis sicca dogs after treatment with CsA-E. Figures are absolute numbers (and %) unless otherwise stated

Parameters/Grades	T0 (n=96)	T1 (n=96)	P Value[a]	T2 (n=60)	P Value[b]
STT-1, mm/min			P<0.001		P<0.001
<10	73 (76)	12 (13)		8 (13)	
10-15	23 (24)	35 (36)		21 (35)	
>15	0	49 (51)		31 (52)	
Conjunctival hyperemia			P<0.001		P<0.001
Grade 0	1 (1)	64 (67)		51 (85)	
Grade 1	27 (28)	28 (29)		9 (15)	
Grade 2	47 (49)	4 (4)		0	
Grade 3	21 (22)	0		0	
Mucous discharge			P<0.001		P<0.001
Grade 0	1 (1)	40 (42)		45 (75)	
Grade 1	23 (24)	51 (53)		14 (23)	
Grade 2	44 (46)	4 (4)		1 (2)	
Grade 3	28 (29)	1 (1)		0	
Corneal pigmentation			P=0.002		P=0.012
Grade 0	53 (55)	63 (66)		39 (65)	
Grade 1	20 (21)	17 (18)		13 (22)	
Grade 2	6 (6)	13 (13)		8 (13)	
Grade 3	17 (18)	3 (3)		0	
Corneal neovascularisation			P<0.001		P<0.001
Grade 0	3 (4)	22 (31)		33 (55)	
Grade 1	23 (32)	36 (50)		24 (40)	
Grade 2	24 (33)	14 (19)		3 (5)	
Grade 3	22 (31)	0		0	
Blepharospasm			P<0.001		P<0.001
Grade 0	51 (53)	85 (89)		60 (100)	
Grade 1	27 (28)	10 (10)		0	
Grade 2	15 (16)	1 (1)		0	
Grade 3	3 (3)	0		0	

CsA-E: Cyclosporine A diluted in vitamin E; STT-1: Schirmer tear test 1; T0: baseline; T1; 3 months follow-up; T2; 6 months follow-up: Grade 0: Not affected; Grade 1: Mildly affected; Grade 2: Moderately affected; and Grade 3: Severely affected: [a]Non-parametric Wilcoxon test between T0 and T1: [b]Non-parametric Friedman test between T0, T1, and T2.

observed for mucous discharge, corneal pigmentation, corneal neovascularization, and blepharospasm (Table 3). For all those CTR parameters, the difference between T0 and T1 and between T0, T1 and T2 was statistically significant.

Chronic Superficial Keratitis

The baseline characteristics of CSK dogs are summarised in Table 2. Most patients were sprayed females (16, 59%) and their median age was five years (range: 3.0-12 years). German shepherds accounted for 57% of the

Table 4: Evolution during follow-up of different clinical treatment response parameters in chronic superficial keratitis dogs after treatment with CsA-E. Figures are absolute numbers (and %)

Parameters/Grades	T0 (n=27)	T1 (n=27)	P Value[a]	T2 (n=14)	P Value[b]
Conjunctival hyperemia			P<0.001		P=0.007
Grade 0	10 (37)	27 (100)		14 (100)	
Grade 1	13 (48)	0		0	
Grade 2	4 (15)	0		0	
Grade 3	0	0		0	
Mucous discharge			P=1		P=1
Grade 0	27 (100)	27 (100)		14 (100)	
Grade 1	0	0		0	
Grade 2	0	0		0	
Grade 3	0	0		0	
Corneal pigmentation			P<0.001		P=0.007
Grade 0	0	1 (4)		0	
Grade 1	7 (26)	19 (70)		10 (71)	
Grade 2	9 (33)	6 (22)		4 (29)	
Grade 3	11 (41)	1 (4)		0	
Corneal neovascularisation			P<0.001		P<0.001
Grade 0	0	9 (33)		9 (64)	
Grade 1	6 (22)	17 (63)		4 (36)	
Grade 2	10 (37)	0		0	
Grade 3	11 (41)	1 (4)		0	
Blepharospasm			P=1		P=1
Grade 0	27 (100)	27 (100)		10 (100)	
Grade 1	0	0		0	
Grade 2	0	0		0	
Grade 3	0	0		0	
Corneal infiltrates			P<0.001		P<0.001
Grade 0	0	24 (89)		13 (93)	
Grade 1	6 (22)	3 (11)		0	
Grade 2	17 (63)	0		1 (7)	
Grade 3	4 (15)	0		0	

CsA-E: Cyclosporine A diluted in vitamin E; T0: baseline; T1; 3 months follow-up; T2; 6 months follow-up: Grade 0: Not affected; Grade 1: Mildly affected; Grade 2: Moderately affected; and Grade 3: Severely affected: [a]Non-parametric Wilcoxon test between T0 and T1: [b]Non-parametric Friedman test between T0, T1 and T2.

Table 5: Evolution during follow-up of different clinical treatment response parameters in proliferative eosinophilic keratitis cats after treatment with CsA-E

Parameters/Grades	T0 (n=23)	T1 (n=23)	P Value[a]	T2 (n=17)	P Value[b]
Conjunctival hyperemia			P=0.016		P<0.009
Grade 0	5 (22)	22 (96)		17 (100)	
Grade 1	14 (61)	1 (4)		0	
Grade 2	3 (13)	0		0	
Grade 3	1 (4)	0		0	
Mucous discharge			P=0.073		P=0.031
Grade 0	15 (65)	23 (100)		17 (100)	
Grade 1	6 (26)	0		0	
Grade 2	2 (9)	0		0	
Grade 3	0	0		0	
Corneal neovascularisation			P=0.014		P=0.003
Grade 0	0	5 (22)		14 (82)	
Grade 1	6 (26)	18 (78)		3 (28)	
Grade 2	15 (65)	0		0	
Grade 3	2 (9)	0		0	
Blepharospasm			P=0.83		P=0.135
Grade 0	19 (83)	23 (100)		17 (100)	
Grade 1	4 (17)	0		0	
Grade 2	0	0		0	
Grade 3	0	0		0	
Corneal infiltrates			P=0.019		P=0.003
Grade 0	0	14 (61)		17 (100)	
Grade 1	7 (30)	9 (39)		0	
Grade 2	9 (61)	0		0	
Grade 3	2 (9)	0		0	

Figures are absolute numbers (and %)CsA-E: Cyclosporine A diluted in vitamin E; T0: baseline; T1: 3 months follow-up; T2: 6 months follow-up. Grade 0: Not affected; Grade 1: Mildly affected; Grade 2: Moderately affected and Grade 3: Severely affected. [a]Non-parametric Wilcoxon test between T0 and T1. [b]Non-parametric Friedman test between T0, T1 and T2.

total CSK cases. At T0, thirteen (48%) dogs presented grade 1 and four (15%) grade 2 conjunctival hyperaemia (Table 4). At T1, this symptom had resolved in all our patients (P<0.001), which was maintained in our T2 sample (P<0.001). None of our CSK patients presented mucous discharge or blepharospasm at any of the follow-up times. Most of our patients (11, 41%) showed a grade 3 corneal pigmentation at T0, but at T1, only one (4%) was classified as grade 3 and most of them presented a grade 1 (19, 70%) or grade 2 (6, 22%) corneal pigmentation (P<0.001). These proportions remained similar in our T2 sample (P=0.007). Regarding corneal neovascularization, the majority of our cases (10, 37%) presented a grade 3 at T0, but at T1, most of them (17, 63%) were classified as grade 1 (P<0.001). The number of grade 0 patients increased from zero at T0 to nine (33%) at T1 and remained at nine (64%) at T2 (P<0.001). Concerning corneal infiltrates, most of our patients presented a grade 2 (17, 63%) at T0, whereas at T1, only three (11%) patients were classified as grade 1, the rest (24, 89%) were considered grade 0 (P<0.001). At T2, only one (7%) patient showed a grade 2 and the rest (13, 93%) were classified as grade 0 (P<0.001) (Table 4).

Proliferative Eosinophilic Keratitis

The baseline characteristics of PEK cats are summarised in Table 2. Most patients were intact males (13, 57%) and their median age was four years (range: 1.0-16 years). At T0, 18 (78%) cats presented some degree (grade 1 or above) of conjunctival hyperaemia (Table 5). At T1, only one (4%) patient presented some degree of this symptom (P=0.016). None of the patients in our T2 sample presented conjunctival hyperaemia (P<0.009). Only a small proportion (8, 35%) of our PEK patients presented some degree of mucous discharge at T0, but they were all classified as grade 0 at T1 (17, 100%) (P=0.073) and at T2 (P=0.031). The same was found for blepharospasm, although with different proportions. All patients presented some degree of corneal neovascularization at T0, but most were classified as grade 1 (18, 78%) at T1 (P=0.014) and as grade 0 (14, 82%) at T2 (P=0.003). Regarding corneal infiltrates, all patients presented a grade 1 or above at T0, whereas at T1 most of them (14, 61%) were classified as grade 0 (P=0.019) and at T2 they were all grade 0 (P=0.003) (Table 5).

Safety and Tolerability

No systemic adverse events were detected, but two dogs and one cat (5 eyes) showed conjunctival hyperaemia and blepharospasm at T1, which prompted a switch of the immunomodulatory drug.

DISCUSSION

To the best of our knowledge, this was the first study to evaluate vitamin E as an ophthalmic adjuvant of CsA in animals. We demonstrated that vitamin E was an effective, safe, and well-tolerated vehicle for CsA in dogs with KCS and CSK and in cats with PEK.

As expected, brachycephalic dogs were highly represented among our KCS cases since these breeds have been demonstrated to be inclined to present KCS (Barrett et al. 1991; Williams 2008). In our study, the treatment of KCS with CsA-E was effective to reduce all baseline CTR parameters at three-month follow-up. The improvement in the grading scale for all symptoms was more marked at the six-month follow-up. Importantly, tear production levels were restored in most dogs already after three months of treatment. Our results are in agreement with previous reports demonstrating the lacrimostimulant effect of CsA (Moore et al. 2001; Dodi 2015). In addition, six months after starting CsA-E, no patient presented severe corneal pigmentation and neovascularization, and only a few of them showed a moderate degree of these symptoms. These results are in contrast with previous evidence describing corneal pigmentation and neovascularization resolution beyond 12 months in KCS dogs treated with CsA (Kaswan 1994).

Regarding CSK, a high prevalence in German shepherds was found in our study, as previously reported (Chavkin et al. 1994; Jokinen et al. 2011). Our results showed an improvement in all response parameters after treatment with CsA-E, and corneal pigmentation and neovascularization, in particular, showed significant differences six months after treatment. However, since none of our patients presented neither mucous discharge nor blepharospasm at baseline, no conclusions could be drawn regarding these symptoms. Notably, although none of our patients exhibited moderate corneal infiltrates at the three-month follow-up, one dog was classified as such six months after CsA-E treatment. This might be due to owner noncompliance in drug administration. The results of our study are in agreement with previous studies demonstrating the efficacy of CsA in the treatment of CSK (Jackson et al. 1991; Williams et al. 1995). Other immunosuppressants, such as tacrolimus and pimecrolimus, have been found effective in treating CSK (Nell et al. 2005; Balicki and Trbolova 2010). However, these studies used corn oil (known to cause side effects) (de Oliveira and Wilson 2019) or sodium chloride (not suitable to the lipophilic nature of CsA) as vehicles. Our results with CsA-E showed that not only vitamin E could enhance CsA properties but also that these other immunosuppressants could benefit from this alternative solvent. The impact of vitamin E could be explained by its effect on similar pathologies, since some studies in rats and rabbits have demonstrated its efficacy in treating cataracts and keratocyte apoptosis after refractive surgery (Bilgihan et al. 2001; Kojima et al. 1996; Nagata et al. 1999).

In relation to PEK, our study showed that, globally, all clinical signs showed a significant improvement three months after CsA-E treatment and nearly all completely remitted at the six-month follow-up. The results were not statistically significant for mucous discharge and blepharospasm because of the low number of patients presenting these symptoms at baseline. Therapeutic management of PEK cats usually consists of topical corticosteroids, although relapses after treatment cessation and, in some cases, exacerbation of existing infections have been reported (Stiles and Coster 2016). CsA has been demonstrated to effectively treat PEK in most cats, although unresponsiveness was also reported (Dean and Meunier 2013; Stiles and Coster 2016). Therefore, alternative treatments have been found, including topical megestrol acetate (Villatoro et al. 2018) and adipose-derived mesenchymal stromal cells (Dixon et al. 2018), albeit not devoid of drawbacks themselves. CsA-E

represents a different option and is yet another alternative to be added to the toolbox in the management of feline PEK. In this case, the potential synergistic effect of vitamin E could be due to its anti-inflammatory properties. A previously reported study in rabbits demonstrated that vitamin E could be used effectively as a diffusion barrier for pirfenidone-loaded contact lenses to treat inflammation after alkali burns (Parrilha et al. 2015).

In our study, three adverse events were reported. However, topical CsA is known to produce adverse reactions in humans (Bilgihan et al. 2001), dogs (Gregory et al. 1989; Kaswan and Salisbury 1990; Radziejewski and Balicki 2016) and cats (Gilger and Allen 1998; Stiles and Coster 2016; Paradiso et al. 2016). Taking that into account, the adverse events we observed might have been caused by CsA rather than vitamin E. Previous reports have shown no irritant adverse effect of vitamin E (De Campos et al. 2001; Caruso et al. 2020a; Caruso et al. 2020b), in sharp contrast with other oil solvents often used for ophthalmic preparations (Lallemand et al. 2003; Guo et al. 2015). Altogether, our results indicate that vitamin E could be considered a safe and tolerable vehicle for topical CsA.

The main limitation of the present study was the absence of a control group, which precludes a proper comparison. Besides, although the sample size was bigger than those of some related studies (Kaswan et al. 1989; Moore et al. 2001; Nell et al. 2005; Balicki and Trbolova 2010; Villatoro et al. 2018), it could be globally considered small. Although not all patients received concurrent treatments, we could not rule out the beneficial effects those may have had on the patient, which we attributed to CsA-E. Also, the lost to follow-up of 55 animals at the six-month follow-up hampers a correct correlation from baseline. Finally, the minimum threshold of three months of follow-up could have excluded patient's intolerant to CsA-E who showed clinical signs before three months.

Conclusion

Our study showed that CsA-E was an effective treatment for KCS and CSK in dogs and PEK in cats by improving patients' clinical response up to six months after treatment. In addition, vitamin E was deemed safe and tolerable for its topical use in dogs and cats since the few adverse events we observed could have been attributed to CsA. Although more extensive studies are needed to validate or refute our results, vitamin E could be considered an adequate alternative to other oil-based solvents, which tend to be more irritant, when considering CsA ophthalmic preparations.

Author's Contribution

KA (First author): contribution to the design of the study, interpretation of data, revising intellectual content and final approval of the final version. Agreement to be accountable for all aspects of the work related to the accuracy or integrity of any part is appropriately investigated and resolved. EA: data collection and final approval of the final version to be published. MC: Revising intellectual contents and final approval of the version to be published.

Acknowledgement

The authors thank Matías Rey-Carrizo, PhD, for providing editorial support.

REFERENCES

Acheampong A, Shackleton M, Lam S, Rudewicz P and Tang-liu D, 1998. Cyclosporine distribution into the and systemic blood following topical dosing of cyclosporine to rabbit, dog, and human eyes. Advances in Experimental Medicine and Biology 438: 1001–1004. https://doi.org/10.1007/978-1-4615-5359-5_144

Ayala M and Söderberg PG, 2004. Vitamin E can protect against ultraviolet radiation-induced cataract in Albino rats. Ophthalmic Research 36: 264-269. https://doi.org/10.1159/000081206

Balicki I and Trbolova A, 2010. Clinical evaluation of tacrolimus eye drops for chronic superficial keratitis treatment in dogs. Bulletin of the Veterinary Institute Pulawy: 54: 251-258.

Barrett P, Scagliotti R, Merideth R, Jackson P and Alarcon F, 1991. Absolute corneal sensitivity and corneal trigeminal nerve anatomy in normal dogs. Progress in Veterinary & Comparative Ophthalmology 1: 245-254.

Bilgihan K, Adiguzel U, Sezer C, Akyol G and Hasanreisoglu B, 2001. Effects of topical vitamin E on keratocyte apoptosis after traditional photorefractive keratectomy. Ophthalmologica 215: 192-196. https://doi.org/10.1159/000050857

Caruso C, Eletto D, Rinaldi M, Pacente L, Troisi S, Semeraro F, Dell'Omo R and Costagliola C, 2020a. Effectiveness and safety of topical chlorhexidine and vitamin E TPGS in the treatment of acanthamoeba keratitis: A survey on 29 cases. Journal of Clinical Medicine 9: 3775. https://doi.org/10.3390/jcm9113775

Caruso C, Porta A, Tosco A, Eletto D, Pacente L, Bartollino S and Costagliola C, 2020b. A novel vitamin E TPGS-based formulation enhances chlorhexidine bioavailability in corneal layers. Pharmaceutics 12: 642. https://doi.org/10.3390/pharmaceutics12070642

Chavkin M, Roberts S, Salman M, Severin G and Scholten N, 1994. Risk factors for development of chronic superficial keratitis in dogs. Journal of the American Veterinary Medical Association 204: 1630-1634.

De Campos AM, Sánchez A and Alonso MJ, 2001. Chitosan nanoparticles: A new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A. International Journal of Pharmaceutics 224: 159-168. https://doi.org/10.1016/s0378-5173(01)00760-8

De Oliveira RC and Wilson SE, 2019. Practical guidance for the use of cyclosporine ophthalmic solutions in the management of dry eye disease. Clinical Ophthalmology 13: 1115-1122. https://doi.org/10.2147/OPTH.S184412

Dean E and Meunier V, 2013. Feline eosinophilic keratoconjunctivitis: A retrospective study of 45 cases (56 eyes). Journal of Feline Medicine and Surgery 15: 661-666. https://doi.org/10.1177/1098612X12472181

Dixon P, Ghosh T, Mondal K, Konar A, Chauhan A and Hazra S, 2018. Controlled delivery of pirfenidone through vitamin E-loaded contact lens ameliorates corneal inflammation. Drug Delivery and Translational Research 8: 1114-1126. https://doi.org/10.1007/s13346-018-0541-5

Dodi P, 2015. Immune-mediated keratoconjunctivitis sicca in dogs: current perspectives on management. Veterinary Medicine: Research and Reports 6: 341-347. https://doi.org/10.2147/VMRR.S66705

Gilger BC and Allen JB, 1998. Cyclosporine A in veterinary ophthalmology. Veterinary Ophthalmology 1: 181-187. https://doi.org/10.1046/j.1463-5224.1998.00039.x

Gregory CR, Hietala SK, Pedersen NC, Gregory TA, Floyd-Hawkins KA and Patz JD, 1989. Cyclosporine Pharmacokinetics in Cats Following Topical Ocular Administration. Transplantation 47: 516-519. https://doi.org/10.1097/00007890-198903000-00023

Guo C, Zhang Y, Yang Z, Li M, Li F, Cui F, Liu T, Shi W and Wu X, 2015. Nanomicelle formulation for topical delivery of cyclosporine A into the cornea: In vitro mechanism and in vivo permeation evaluation. Scientific Reports 5: 12968. https://doi.org/10.1038/srep12968

Jackson P, Kaswan R, Merideth R and Barrett P, 1991. Chronic superficial keratitis in dogs: a placebo controlled trial of topical cyclosporine treatment. Progress in Veterinary & Comparative Ophthalmology 1: 269-275.

Jokinen P, Rusanen EM, Kennedy LJ and Lohi H, 2011. MHC class II risk haplotype associated with Canine chronic superficial keratitis in German Shepherd dogs. Veterinary Immunology and Immunopathology 140: 37-41. https://doi.org/10.1016/j.vetimm.2010.11.007

Kaswan R, 1994. Characteristics of a Canine Model of KCS: Effective Treatment with Topical Cyclosporine. In: Lacrimal Gland, Tear Film, and Dry Eye Syndromes. Advances in Experimental Medicine and Biology 1994: 583-594.

Kaswan RL and Salisbury MA, 1990. A new perspective on canine keratoconjunctivitis sicca: Treatment with ophthalmic cyclosporine. Veterinary Clinics of North America: Small Animal Practice 20: 583-613. https://doi.org/10.1016/s0195-5616(90)50052-2

Kaswan RL, Salisbury M and Ward DA, 1989. Spontaneous canine keratoconjunctivitis sicca. Archives Ophtalmology 107: 1210-1216.

Kojima M, Shui YB, Murano H and Sasaki K, 1996. Inhibition of steroid-induced cataract in rat eyes by administration of vitamin-E ophthalmic solution. Ophtalmic Research 28: 64-71. https://doi.org/10.1159/000267958

Labbé A, Baudouin C, Ismail D, Amrane M, Garrigue JS, Leonardi A, Figueiredo FC, Van Setten G and Labetoulle M, 2017. Pan-European survey of the topical ocular use of cyclosporine A. Journal Français D'Ophtalmologie 40: 187-195. https://doi.org/10.1016/j.jfo.2016.12.004

Lallemand F, Felt-Baeyens O, Besseghir K, Behar-Cohen F and Gurny R, 2003. Cyclosporine A delivery to the eye: A pharmaceutical challenge. European Journal of Pharmaceutics and Biopharmaceutics 56: 307-318. https://doi.org/10.1016/s0939-6411(03)00138-3

Miyazawa T, Burdeos GC, Itaya M, Nakagawa K and Miyazawa T, 2019. Vitamin E: Regulatory Redox Interactions. IUBMB Life 71: 430-441. https://doi.org/10.1002/iub.2008

Moore CP, McHugh JB, Thorne JG and Phillips TE, 2001. Effect of cyclosporine on conjunctival mucin in a canine keratoconjunctivitis sicca model. Investigative Ophthalmology & Visual Science 42: 653-659.

Mustacich DJ, Bruno RS and Traber MG, 2017. Vitamin E. Vitamins and Hormones 76: 1-21. https://doi.org/10.1016/S0083-6729(07)76001-6

Nagata M, Kojima M and Sasaki K, 1999. Effect of vitamin E eye drops on naphthalene-induced cataract in rats. Journal of Ocular Pharmacology and Therapeutics 15: 345-350. https://doi.org/10.1089/jop.1999.15.345

Nell B, Walde I, Billich A, Vit P and Meingassner JG, 2005. The effect of topical pimecrolimus on keratoconjunctivitis sicca and chronic superficial keratitis in dogs: Results from an exploratory study. Veterinary Ophthalmology 8: 39-46. https://doi.org/10.1111/j.1463-5224.2005.04062.x

Paradiso P, Serro AP, Saramago B and Chauhan A, 2016. Controlled release of antibiotics from vitamin E loaded silicone-hydrogel contact lenses. Journal of Pharmaceutical Sciences 105: 1164-1172. https://doi.org/ 10.1016/S0022-3549(15)00193-8

Parrilha LR, Nai GA and Giuffrida R, Barbero RC, Padovani LDF, Pereira RHZ, Silva DA, Silva MCA, Deniz MS and Andrade SF, 2015. Comparison of 1% cyclosporine eye drops in olive oil and in linseed oil to treat experimentally-induced keratoconjunctivitis sicca in rabbits. Open Access Arquivos Brasileiros de Oftalmologia 78: 295-299. https://doi.org/10.5935/0004-2749.20150078

Peh HY, Tan WSD, Liao W and Wong WSF 2016. Vitamin E therapy beyond cancer: Tocopherol versus tocotrienol. Pharmacology & Therapeutics 162: 152-169. https://doi.org/10.1016/j.pharmthera.2015.12.003

Pekmezci D, 2011. Vitamin E and Immunity. Vol 86. 1st Ed. Elsevier Inc.

Radziejewski K and Balicki I, 2016. Comparative clinical evaluation of tacrolimus and cyclosporine eye drops for the treatment of canine keratoconjunctivitis sicca. Acta Vetinaria Hungarica 64: 313-329. https://doi.org/10.1556/004.2016.030

Read R, Barnett K and Sansom J, 1995. Cyclosporine-responsive keratoconjunctivitis in the cat and horse. Veterinary Record 137: 170-171. https://doi.org/10.1136/vr.137.7.170

Spiess AK, Sapienza JS and Mayordomo A, 2009. Treatment of proliferative feline eosinophilic keratitis with topical 1.5% cyclosporine: 35 cases. Veterinary Ophthalmology 12: 132-137. https://doi.org/10.1111/j.1463-5224.2008.00679.x

Stiles J and Coster M, 2016. Use of an ophthalmic formulation of megestrol acetate for the treatment of eosinophilic keratitis in cats. Veterinary Ophthalmology 19: 86-90. https://doi.org/10.1111/vop.12371

Villatoro AJ, Claros S, Fernández V, Alcoholado C, Fariñas F, Morena A, Becerra J and Andrades JA, 2018. Safety and efficacy of the mesenchymal stem cell in feline eosinophilic keratitis treatment. BMC Veterinary Research 14: 1-13. https://doi.org/10.1186/s12917-018-1413-4

Whitcup SM, Chan CC, Luyo DA, Bo P and Li Q, 1996. Topical cyclosporine inhibits mast cell-mediated conjunctivitis. Investigative Ophthalmology & Visual Science 37: 2686-2693.

Williams DL, 1997. A comparative approach to topical cyclosporine therapy. Eye 11: 453-464. https://doi.org/10.1038/eye.1997.126

Williams DL, Hoey AJ and Smitherman P, 1995. Comparison of topical cyclosporin and dexamethasone for the treatment of chronic superficial keratitis in dogs. Veterinary Record 137: 635-639.

Williams DL, 2008. Immunopathogenesis of keratoconjunctivitis sicca in the dog. Veterinary Clinics of North America - Small Animal Practice 38: 251-268. https://doi.org/10.1016/j.cvsm.2007.12.002

Xin J, Tang J, Bu M, Sun Y, Wang X, Wu L and Liu H, 2016. A novel eye drop of alpha tocopherol to prevent ocular oxidant damage: Improve the stability and ocular efficacy. Drug Development and Industril Pharmacy 42: 525-534. https://doi.org/10.3109/03639045.2015.1082582