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Original article

Clinical study on the application of dexamethasone and cyclosporine/dimethyl sulfoxide combination eye drops in the initial therapy of chronic superficial keratitis in dogs

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Abstract

Purpose: To assess the initial therapy of chronic superficial keratitis (CSK) in dogs with the use of dexamethasone and cyclosporine/ dimethyl sulfoxide combination eye drops. **Methods:** The study was conducted on 41 dogs – 16 males and 25 females, aged 2 to 9 years, diagnosed with CSK. The disease was treated with two kinds of eye drops containing 0.1% dexamethasone and 0.75% cyclosporine in combination with 30% DMSO, administered three times a day. Prior to the treatment and after 5 weeks of therapy, depigmentation of the third eyelid margin, corneal neovascularization and pigmentation were assessed. The percentage of the corneal surface afflicted with inflammatory processes was calculated with the use of IsoCalc.com's Get Area software for CorelDRAW12. **Results:** The administered therapy resulted in a significant decrease in the mean number of quadrants affected by corneal neovascularization in the right eye from 2.63 prior to treatment to 0.24 after treatment ($p<0.001$), and the left eye from 2.66 to 0.59 ($p<0.001$), respectively. Mean corneal surface afflicted with inflammatory processes was statistically significantly reduced from 53.5% to 26.3% ($p<0.001$) in the case of right corneas, and from 54.5% to 30.2% ($p<0.001$) in the case of left corneas. Of 77 corneas diagnosed with pigmentation, pigmentation reduction was observed in 54 and pigmentation increase in 27. **Conclusions:** Using dexamethasone and cyclosporine/DMSO combination eye drops proved to be a viable initial therapy against CSK, which facilitates reduction of inflammatory processes and neovascularization atrophy, but in many cases does not inhibit the progress of pigmentation.

Key words: chronic superficial keratitis, German Shepherd, cyclosporine, DMSO

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Introduction

Chronic superficial keratitis (CSK) is an idiopathic non-ulcerative common corneal disease It is characterized by a progressive lymphoplasmacytic infiltration in the anterior corneal stroma (Williams 2005). Although exact causes of CSK are still unknown, immune-mediated aetiology is suspected (Jokinen et al. 2011). The most common breed with CSK predisposition is the German Shepherd dog (82 %) but the disease also occurs in other breeds and mixed breed dogs (Slatter et al. 1977). It has been proven that exposure to UV light influences the development of CSK (Chandler et al. 2008, Denk et al. 2011). Inflammatory infiltration and vascularization followed by pigmentation are typical lesions in CSK. A cornea affected with CSK shows pink fibrovascular tissue (Balicki 2010, Balicki 2012). The main symptom of this disease is depigmentation of the nictitating membrane margin and more rarely medial central third eyelid erosion or thickening (Balicki 2010, Balicki 2012).

The response to topical immunosuppressive therapy is a sign of the immune-mediated character of the CSK. Immunomodulatory agents and corticosteroid drops or ointments are topical drugs recommended in CSK patients. Cyclosporine (CsA) eye drops are the treatment of choice but at the beginning of the therapy, both CsA and dexamethasone are recommended. The frequency of the eye drops or ointment administration into the conjunctival sack can be reduced in the course of the therapeutic process, starting with 4-6 times a day for 7-10 days, and slowly reduced over 6-8 weeks with constant monitoring of the status of the patient with progressively excluding dexamethasone and leaving cyclosporine eye drops twice daily.

Dimethyl sulfoxide (DMSO) increases the penetration of substances through biological membranes. DMSO is an organic solvent easily mixed with water, lipids and organic agents. It is a useful vehicle for transporting pharmaceuticals into biological systems thanks to its ability to penetrate skin and other membranes (Rawls et al. 2017). Studies into membrane permeability and carrier effect have been conducted in agriculture, fundamental biology, as well as on animals and humans. Maibach and Feldmann (1967) conducted a study on percutaneous penetration of hydrocortisone and testosterone in DMSO solution. DMSO has not only anti-inflammatory and analgesic qualities but also weak bacteriostatic and diuretic activity, as well as the capacity to remove free radicals (Binnick et al. 1977, Del Maestro et al. 1980, Brayton 1986). Administration of 50% and 60% DMSO increases the healing of the cornea in rabbits (Toczołowski 1992, Kawa 1997). A combination of DMSO and fluconazole in eye drops can be used in fungal keratitis in horses (Balicki 2008). In horses, DMSO was also applied in mares with corneal ulceration due to Listeria monocytogenes and in horses with anterior epithelial defect (Sanchez et al. 2001).

It has also been proven that a combination of dexamethasone and DMSO has better therapeutic action than pure dexamethasone in topical treatment (Balicki 2005). It has also been demonstrated that application of DMSO and cyclosporine for 8 months had no harmful effects on corneal cells (Balicki 2005).

The purpose of the present study was to assess the initial therapy of chronic superficial keratitis in dogs with the use of dexamethasone and cyclosporine/DMSO combination eye drops.

Materials and Methods

The study was conducted on 41 German Shepherd dogs – 16 males and 25 females, aged 2 to 9 years, diagnosed with CSK. The patients underwent detailed ophthalmic examinations using slit-lamp biomicroscopy (Shin Nippon) as well a direct (Welch Allyn) and indirect (Keeler) ophthalmoscopy with a PanOptic ophthalmoscope (Welch Allyn). Schirmer tear tests (Eickemeyer) were also performed.

The dogs were patients of the Department and Clinic of Animal Surgery at the University of Life Sciences in Lublin. The owners were informed about the details of conducted clinical trials and they have given their consent. The study was performed in accordance with the Polish law and with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, Chapter I, Article 1, point 5(b). The research was also approved by the Scientific Research Committee of the Department and Clinic of Animal Surgery at the University of Life Sciences in Lublin (#7/2018) concerning non-experimental clinical patients.

The dogs diagnosed with CSK were treated with two types of ophthalmic drops containing 0.1% dexamethasone (Dexamethason; Polfa Warszawa) and 0.75% cyclosporine in combination with 30% DMSO, administered three times a day. The drug was formulated by a specialist in the field of ophthalmic pharmacy on the basis of a 0.9% NaCl solution. In cases where mucopurulent exudate was present in the conjunctival sac, the therapy was used in conjunction with 0.3% ciprofloxacin (Ciloxan; Alcon) ophthalmic drops administered five times a day for the first 5 days, and then three times a day up to the 14th day of the treatment. Photographic documentation was taken in all patients. Prior to and 5 weeks after the therapy, depigmentation

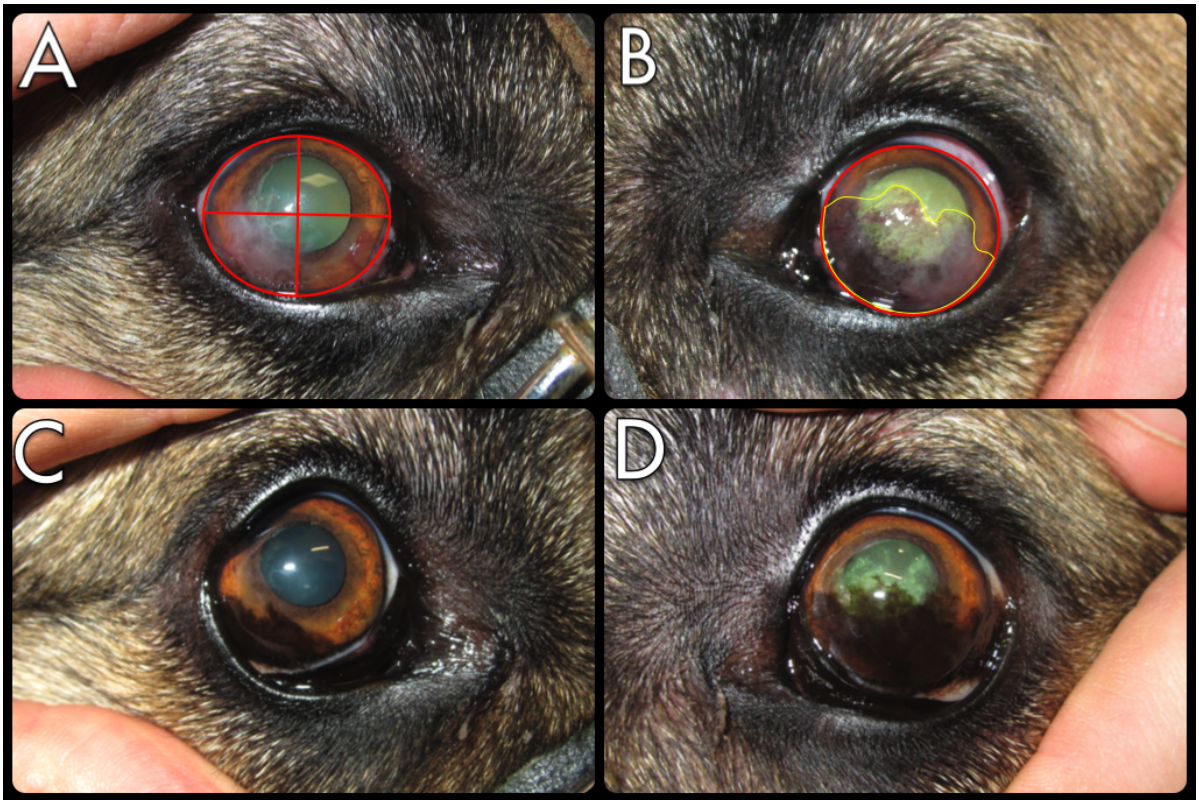

Fig. 1. Dog no. 13. Right cornea prior - A to the 5 week treatment - C, respectively left cornea - B and D. Reduction of neovascularization, fibrovascular infiltration, development of pigmentation. A - example of division of the cornea into quadrants, B - example of designation of the diseased surface of the cornea for calculations.

of the third eyelid margin, corneal area surface affected by inflammatory process, as well the incidence of pigmentation, corneal vascularization, and ocular discharge were determined. The corneas were evaluated relative to their respective quadrants: superonasal, superotemporal, inferonasal and inferotemporal (Fig. 1A). Blood vessel ingrowth into the each corneal quadrant was also assessed and counted. The percentage of the corneal area involved in the inflammatory process including symptoms such as. neovascularization, fibrous tissue infiltration, superficial macular opacities and pigmentation, was calculated on the basis of photographs with the use of IsoCalc.com's Get Area software for Corel DRAW 12 (Fig. 1B).

The corneal pigmentation was evaluated on the basis of its decrease or development, and density reduction, as well as a calculation of the corneal quadrants affected by the pigmentation prior to and 5 weeks after the treatment in all the patients.

The assessment of purulent conjunctivitis was based on the amount of discharge from the conjunctival sac according to a five-point scale: 0 – no eye discharge, 1 – small amount of mucous exudate in the conjunctival sac slight, 2 – discharge at medial canthus, 3 – purulent exudate present along the lower eyelid margin, 4 – purulent exudate present on the eyelids and in the vicinity of the eye socket.

The results were analyzed statistically. The Kolmogorov-Smirnov test revealed a normal distribution of data, therefore the t-Student test was used to compare the parameters before and after the 5-week treatment. Statistical analyses were performed by calculating the arithmetic means and standard deviation (SD) using Statistica12 software (TIBCO Software Inc., USA). The chi-square test was used to compare the total number of corneal quadrants involved in the neovascularization. The significance of differences was set at $p<0.01$ and $p<0.001$.

Results

Pathognomonic symptoms of CSK were observed in all the dogs: corneal vascularization, infiltration of fibro-granulation tissue, and pigmentation (Table 1, 2, 3). Schirmer's test results exceeded 20 mm in all the animals.

The administration of eye drops containing DMSO combined with cyclosporine into the conjunctival sac was well tolerated by the dogs. In 18 animals, immediately after the drug administration, slight closing of the lid slit was observed, but subsided within 5 minutes. After the first 7 days of receiving the drug, the symptom persisted in only 4 dogs.

Table 1. Assessment of corneal lesions responses to dexamethasone and cyclosporine/DMSO therapy.

Case	Age	Sex	Eye	Depigmentation of the third eyelid margin		Corneal neovascularization (number of quadrants)		Corneal area affected (%)		Purulent conjunctivitis	
				Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
1	4	♂	OD	D	R	0	0	18	10	1	0
			OS	D	R	0	0	7	1	2	1
2	6	♂	OD	d	R	4	0	87	47	2	0
			OS	d	R	3	0	74	29	2	0
3	8	♀	OD	d	R	4	0	98	82	2	0
			OS	-	-	4	0	98	49	2	0
4	9	♂	OD	D	r	1	0	25	0	2	1
			OS	D	r	2	0	35	5	2	1
5	5	♂	OD	-	-	2	0	84	46	0	0
			OS	-	-	1	0	67	45	0	0
6	9	♂	OD	D	R	1	0	7	0	3	3
			OS	D	R	2	0	31	25	3	3
7	5	♀	OD	-	-	3	0	60	9	2	0
			OS	-	-	3	0	38	0	2	0
8	6	♀	OD	d	R	1	0	28	25	3	1
			OS	D	R	1	0	16	14	3	1
9	4	♂	OD	-	-	2	0	30	0	2	0
			OS	-	-	4	0	90	50	2	0
10	8	♀	OD	D	R	4	2	100	73	4	2
			OS	D	R	4	2	100	35	4	2
11	5	♀	OD	D	r	4	1	80	26	3	0
			OS	D	r	4	4	100	95	3	1
12	8	♀	OD	d	R	4	0	85	76	2	2
			OS	d	R	4	1	87	76	2	2
13	7	♀	OD	D	R	3	0	45	18	2	2
			OS	D	R	4	0	72	48	2	2
14	8	♂	OD	D	r	3	1	65	25	2	2
			OS	D	r	4	2	80	40	2	2
15	7	♀	OD	-	-	0	0	16	12	3	0
			OS	-	-	0	0	22	17	3	0
16	3	♀	OD	d	R	1	0	24	5	1	0
			OS	d	R	1	0	6	1	1	0
17	3	♂	OD	d	R	4	0	86	39	4	1
			OS	d	R	3	0	56	36	4	1
18	7	♀	OD	D	R	2	0	47	0	2	2
			OS	d	R	1	0	19	4	2	2
19	6	♀	OD	D	D	2	0	23	2	4	3
			OS	D	r	3	1	45	26	4	3
20	8	♀	OD	-	-	4	0	100	95	0	0
			OS	-	-	4	0	83	48	0	0
21	2	♂	OD	D	R	4	0	89	35	1	1
			OS	D	R	4	0	80	25	1	1
22	2	♀	OD	D	R	1	0	15	4	2	1
			OS	D	R	1	0	22	14	2	2
23	4	♂	OD	-	-	4	0	100	77	0	0
			OS	-	-	4	0	100	85	0	0
24	7	♂	OD	d	R	2	0	30	15	1	1
			OS	d	R	3	0	45	10	2	1

cont. Table 1.

Case	Age	Sex	Eye	Depigmentation of the third eyelid margin		Corneal neovascularization (number of quadrants)		Corneal area affected (%)		Purulent conjunctivitis	
				Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
25	6	♀	OD	D	R	4	0	99	60	4	3
			OS	D	R	4	0	96	47	4	3
26	7	♀	OD	-	-	4	0	100	39	2	0
			OS	-	-	4	0	100	44	2	0
27	5	♀	OD	D	r	2	0	29	9	2	1
			OS	D	r	0	0	9	8	2	2
28	2.5	♂	OD	D	R	2	0	15	22	0	0
			OS	D	R	2	0	18	10	0	0
29	5	♂	OD	D	D	3	2	43	35	3	1
			OS	D	D	4	3	90	68	3	1
30	2.5	♀	OD	D	R	2	0	38	12	1	0
			OS	D	R	3	0	43	22	1	0
31	5.5	♀	OD	D	R	3	0	65	27	1	1
			OS	D	R	3	0	44	18	1	1
32	5	♂	OD	D	R	3	0	60	24	1	0
			OS	D	R	4	3	72	48	1	0
33	8	♂	OD	-	-	2	0	37	12	0	0
			OS	-	-	3	0	61	32	0	0
34	2.5	♀	OD	D	R	1	0	20	9	1	0
			OS	D	R	1	0	17	0	1	0
35	6	♂	OD	D	R	4	0	36	15	2	1
			OS	D	R	1	0	22	8	2	1
36	6.5	♀	OD	D	R	1	0	12	6	1	0
			OS	D	R	1	1	15	10	1	0
37	4	♀	OD	D	R	4	0	82	20	2	1
			OS	D	R	4	0	75	60	2	1
38	7	♀	OD	D	R	4	3	100	41	0	0
			OS	D	R	4	3	100	75	0	0
39	6	♀	OD	D	R	3	0	37	10	2	1
			OS	D	R	2	1	46	13	2	1
40	5	♀	OD	D	R	2	0	22	12	3	3
			OS	D	R	1	0	24	6	3	3
41	2.5	♂	OD	D	R	4	1	87	44	3	0
			OS	D	R	4	3	94	42	3	0

Explanation: OD – right eye, OS – left eye, D- depigmentation, d – partial depigmentation, R- repigmentation, r- partial repigmentation

The therapy reduced the corneal reddening in all the patients. Prior to the treatment, symptoms of purulent conjunctivitis were observed in 35 dogs. After the 14-day administration of ciprofloxacin, during the check-up visit after 5 weeks, 3rd degree mucopurulent exudation was evidenced in 4 German Shepherd dogs. In the cases where mucopurulent exudation persisted, it was recommended to perform an antibiogram in cultures collected from the dogs' conjunctival sacs and adapt further treatment to the bacteriological results.

Prior to the therapy, a total of 108 corneal quadrants in right eyes and 109 in left eyes were affected by neovascularization. Neovascularization data are presented in Tables 1 and 2. After 5 weeks of using DMSO and cyclosporine as well as dexamethasone, the number of affected quadrants decreased to 10 and 24, respectively. The mean number of corneal quadrants affected by neovascularization was statistically significantly reduced from 1.63 to 0.24 in right eyes ($p \leq 0.001$) and from 2.66 to 0.59 in left eyes ($p \leq 0.001$). After the 5-week therapy, the inflammatory process was reduced in all corneas diagnosed with CSK (Table 1, 2), (Figs. 1, 2, 3). We were able to demonstrate a statistically significant reduction in the mean surface area of the cornea

Table 2. Area of the affected cornea and corneal quadrants with neovascularization (mean ± SD) and pigmentation before and after a 5-week treatment with dexamethasone and cyclosporine/DMSO combination eye drops.

	Before treatment		After treatment	
	Right cornea	Left cornea	Right cornea	Left cornea
Area of affected cornea (%)	53.5±25.6	54.5±31.2	26.3*±21.0	30.2*±27.0
Corneal quadrants with neovascularization	2.63±0.95	2.66±1.31	0.24*±1.11	0.59*±1.0
Number of all quadrants with neovascularization	108	109	10*	24*
Number of all quadrants with pigmentation	79	76	74	77

Explanation: statistically significant differences * $p \leq 0.001$

Table 3. Changes in the corneal pigmentation.

	Before treatment	After treatment		
	Number of corneas affected	Decrease	Development	Decrease density
Corneal pigmentation	77	54	27	55

affected by the disease – from 53.5% to 26.3% ($p<0.001$) in right eyes and from 54.5% to 30.2% ($p<0.001$) in left eyes.

Prior to the treatment, varying degrees of pigmentation were identified in 38 right and 39 left corneas. After 5 weeks, the area of pigmentation was reduced in 25 and continued to develop in 15 right corneas, with the density of pigmentation reduction and corneal transparence improvement in 26; in left eyes the results were 29, 12 and 29 respectively. In 12 cases, both development and reduction of pigmentation were observed in different areas of the same cornea. During the treatment, pigmentation developed even in patients with minimal pigmentation around the inferotemporal corneal limbus prior to the treatment. This occurred in the case of 2 corneas (dog no. 13 – right cornea – Fig. 1 A, C, dog no. 19 – left cornea – Fig. 3 B, D). The assessment of pigmentation was hindered due to the presence of a thick, fibro-granulation infiltration. If pigmentation was observed after the reduction of inflammatory infiltration, it was considered as pigmentation development. The results pertinent to pigmentation are presented in Tables 2 and 3.

Before the treatment, various degrees of depigmentation of the nictitating membrane margin was observed in 33 dogs, in 65 nictitating membranes. After 5 weeks, repigmentation was observed in all, except for three membranes. In case of one third eyelid the depigmentation progressed during the treatment (dog no. 19 – right nictitating membrane Fig. 3 A, C). Notably, in some cases the repigmentation was significant but not complete, with certain small depigmented area remaining. Such cases were nonetheless considered as repigmentation but marked properly in Table 1.

Discussion

Cornea evaluation based on the aforementioned division into respective quadrants is justified by the specificity of CSK as a disease. In a study conducted by Sanchez et al. (2016), corneas were evaluated with the division into the axial cornea and peripheral cornea, further divided into dorsal, ventral, medial and lateral regions. Given the fact that inflammatory lesions accompanying CSK usually develop in the inferotemporal quadrant, the above classification could lead to a certain adulteration of research results. Therefore, the adopted division entailed drawing out two perpendicular lines through the center of the cornea, effectively dividing it into superonasal, superotemporal, inferonasal and inferotemporal quadrants. This division had also been employed in our previous studies pertaining to CSK treatment, which allows a direct comparison of the effectiveness of respective therapeutic methods (Balicki 2005, Balicki and Trbolova 2010, Balicki 2012)

The use of DMSO in combination with cyclosporine aims not only to strengthen the anti-inflammatory action. DMSO is also used as a vehicle for transporting pharmaceuticals into biological systems (Rawls at al. 2017). In this case, the application of DMSO was intended to facilitate the effects of cyclosporine by improving its capacity to penetrate the corneal tissue. In a previous study, Balicki (2012) described the use of 0.02 % tacrolimus and 50 % DMSO in the form of two separate preparations. Irritative effects of the medicines were observed in 6 of 16 dogs tested, as manifested by their rubbing of the eye socket area. So far, cyclosporine has been used primarily in the form

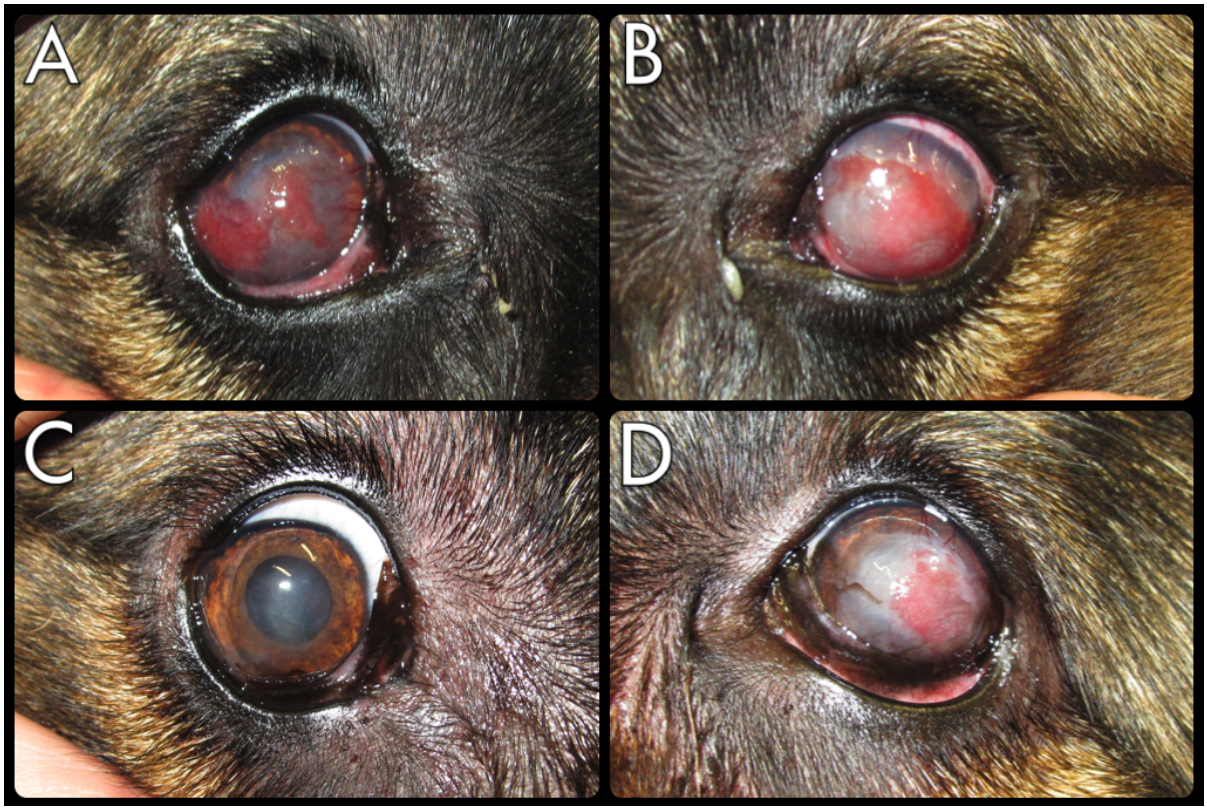

Fig. 2. Dog no. 11. Right cornea before - A and after 5 week treatment - C, respectively left cornea - B and D. Disparity in symptoms and response to the treatment. Total reduction of neovascularization, fibrovascular infiltration in the right cornea and limited improvement in the left cornea.

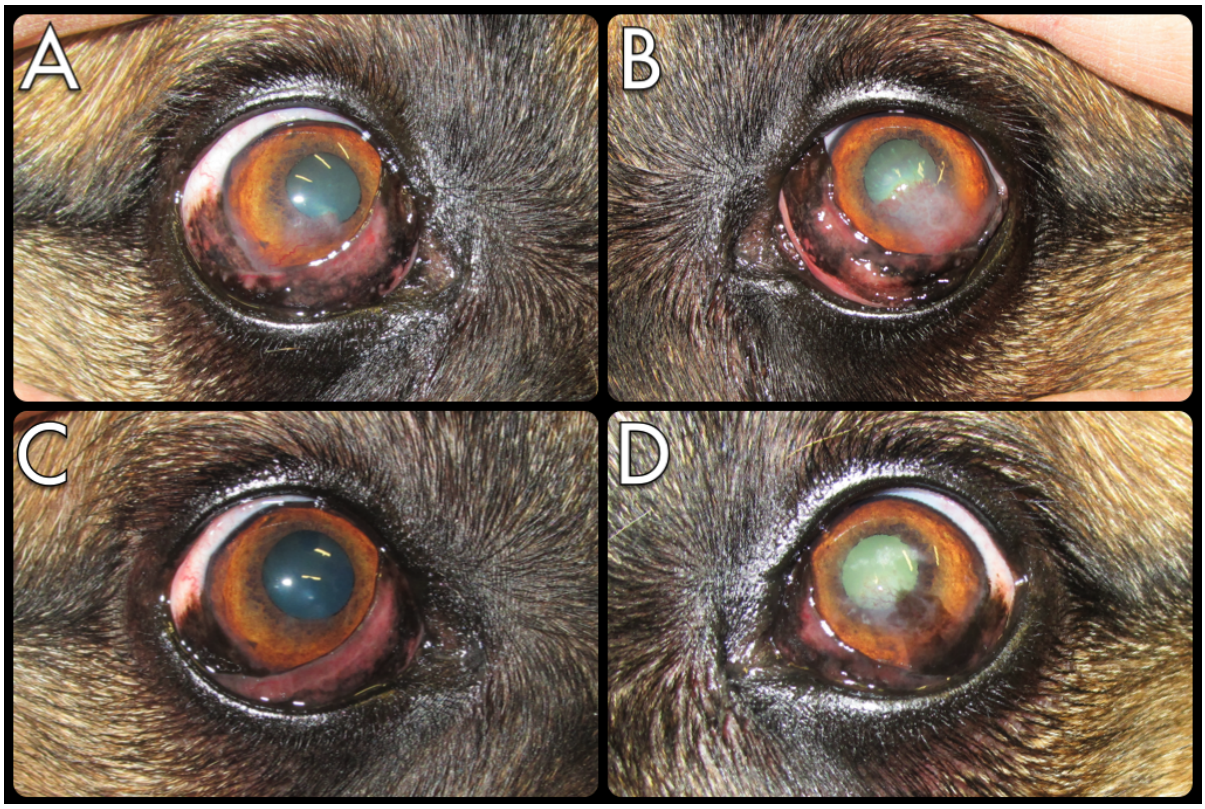

Fig. 3. Dog no. 19. State of depigmentation of the right third eyelid before - A and after 5 week treatment - C; minimal pigmentation around the left inferotemporal corneal limbus prior to the treatment - B and development of the pigmentation after 5 week treatment - D.

of oily solutions (Parrilha et al. 2015). Cyclosporine can be delivered to the conjunctival sack in a form of aqueous drops, but the low solubility of cyclosporine in water limits penetration. As demonstrated by Williams (1997) olive oil or corn oil solutions allowed greater penetration. In the present study, the use of 0.75% cyclosporine with 30% DMSO provided normal saline-based eye drops, hence there was no need for cyclosporine to be dissolved in oil. This allowed minimal irritative reaction to the medicine with the simultaneous maintenance of its satisfactory therapeutic effects. Furthermore, combining the two medicines in a single ophthalmic preparation allowed a lower number of overall medicine administrations necessary.

Both in earlier (Balicki 2012) and the present study, it was demonstrated that CSK is often accompanied by purulent conjunctivitis as a symptom of a bacterial infection not directly related to CSK. In previous research, 81% of the cases were found to include symptoms of significant mucopurulent exudation (Balicki 2012). In the present study, the same were observed in 85% of the patients. Consequently, the treatment had to include simultaneous administration of antibiotic eye drops into the conjunctival sac.

It is assumed that, particularly in the first stage of CSK therapy, the frequency of medicinal preparations administration should be increased (Balicki 2008). Corticosteroids prove crucial at this stage, most often dexamethasone or prednisolone, administered in the form of ophthalmic drops or ointments or, if there is no response to the treatment, as subconjunctival injections. As shown in a study by Williams et al. (1995), administration of dexamethasone for 6 weeks resulted in shrinkage of inflammatory infiltration. Satisfactory and quick therapeutic effects were obtained in the initial phase of treatment by combining cyclosporine with corticosteroids. In studies on the combined use of cyclosporine and dexamethasone, varying degrees of inflammatory infiltration reduction in the treated dogs were reported. The most significant improvement was observed after 2 months of therapy (Balicki 2005). In the present study, after 5 weeks of the treatment a reduction of inflammation was observed in the case of all corneas showing symptoms of CSK. A reduction was observed in terms of the corneal surface affected by inflammatory process in the right and left corneas, respectively by 27.2% and 24.3%. In the study on the use of tacrolimus, the observed decrease was by 25% and 19%, respectively (Balicki and Trbolova 2010), and for tacrolimus combined with DMSO - 26% and 20% (Balicki 2012).

The elimination of vascularization is the most important factor evidencing inhibition of CSK development. Prevention of blood vessel ingrowth is an important outcome of the therapy as it prevents the development of local inflammation foci and pigmentation in the subsequent stage of the disease. Nell et al. (2005) observed a significant reduction in blood vessel ingrowth already after 2 weeks of therapy, and complete elimination thereof after 11 weeks of pimecrolimus administration. In a study on the use of tacrolimus, Balicki and Trbolova (2010) demonstrated complete elimination of vascularization in 39% of right cornea quadrants and 20% of left cornea quadrants already after 4 weeks of the treatment. At the same time, in a therapy entailing the use of tacrolimus combined with DMSO, after 5 weeks the number of quadrants affected by vascularization was decreased in right and left corneas by 42% and 41.7%, respectively (Balicki 2012). In the present study, after 5 weeks of receiving DMSO with cyclosporine and dexamethasone, the number of right and left eye corneal quadrants affected by vascularization was reduced by 90.7% and 77.9%, respectively. This indicates a significantly better therapeutic effect of the combined therapy using eye drops containing both cyclosporine and DMSO. The limited effect of the treatment may be caused mainly by the excessive exposure to sunlight and inadequate administration of drugs by the owners.

The clinical symptom of CSK that proves the most difficult to eliminate is the corneal pigmentation. Difficulties emerge already at the level of assessing its advancement. In the course of CSK, vascularization is accompanied by the development of the fibrous tissue that covers the cornea. This prevents the exact determination of the extent of the pigmentation as it can be located below the layer of inflammatory infiltration. Hence, the preliminary assessment of the extent of the corneal pigmentation is, by necessity, limited to the pigmentation visible during the ophthalmic examination beyond the area affected by the fibrous tissue infiltration. In many cases it has been observed that employed therapy fails to inhibit the development of the pigmentation despite effectively reducing the severity of other symptoms. As confirmed in earlier studies, the reduction of the pigmentation area within the stroma is usually impossible (Balicki and Trbolova 2010, Balicki 2012). In the study on the use of tacrolimus in combination with DMSO (Balicki and Trbolova 2010), the corneal pigmentation was reduced in 43.8% of the cases, and transparence improved in 53.1% of the corneas. In the present study, after 5 weeks of therapy, the pigmentation was reduced in 72.9% and continued to develop in 36.5% of the corneas. In the course of the therapy, the changes in terms of the extent of the pigmentation varied, sometimes even in the context of the same cornea. In a study on the use of cyclosporine and dexamethasone in the course of CSK, Williams et al. (1995) reported no reduction of the extent of the corneal pigmentation but observed improved transparence of

the already existing pigmentation. In the present study, improvement in terms of corneal transparence at the site of the pigmentation was observed in 74.3% of the corneas.

Apart from clinical symptoms related to the cornea, the course of CSK includes the characteristic depigmentation of the nictitating membrane margin. In a study on the use of cyclosporin in cases of corticosteroid-resistant CSK, Bigelbach (1994) reported complete repigmentation of the nictitating membrane only after long-term treatment. Meanwhile, in our study after 5 weeks of the treatment, we observed complete repigmentation in 95.4% of nictitating membranes. This group included 9 membranes where repigmentation was significant, but not complete. In a previous study on the use of tacrolimus in the treatment of CSK, repigmentation was observed in 78.6% of nictitating membranes (Balicki and Trbolova 2010), and in the study on the effectiveness of tacrolimus combined with DMSO (Balicki 2012) repigmentation occurred in 93.8% of nictitating membranes.

The present paper documents the effectiveness of 0.75% cyclosporine combined with 30% DMSO in a water solution and administered as eye drops in the course of initial therapy of chronic superficial keratitis in dogs. The therapy results in more significant and faster reduction of the fibrovascular tissue and corneal vascularization as compared to the results of previous studies (Nell et al. 2005, Balicki 2010, Balicki 2012). The challenge in CSK therapy remains the effective inhibition and reduction of the corneal pigmentation.

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