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Original article

Clinical study on the application of tacrolimus and DMSO in the treatment of chronic superficial keratitis in dogs

I. Balicki

Department and Clinic of Animal Surgery, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Głęboka 30, 20-612 Lublin, Poland

Abstract

The objective of this study was to assess the treatment of chronic superficial keratitis (CSK) in dogs with the use of tacrolimus and DMSO.

The study was conducted on 16 dogs – 7 males and 9 females, aged 3 to 11 years, diagnosed with CSK. The disease was treated with ophthalmic drops containing 0.02% tacrolimus and 50% DMSO, administered to the ocular surface three times a day. Prior to the treatment and after 5 weeks of therapy, the corneal neovascularisation, pigmentation, and also the redness and depigmentation of the third eyelid margin were assessed. The percentage of the corneal surface afflicted with inflammatory processes was calculated on the basis of photographs taken with the use of IsoCalc.com's Get Area software for Corel DRAW 12.

It was found that the application of tacrolimus and DMSO caused a reduction of inflammatory process and neovascularisation in the cornea. The mean corneal surface afflicted with inflammatory processes was statistically significantly reduced from 69.9% to 43.9% ($p \leq 0.01$) – in case of the right corneas, and from 58.9% to 38.6% in case of the left corneas. Of 32 corneas diagnosed with the pigmentation, the reduction of the pigmentation was observed in 14, while in 16 the pigmentation increased.

The treatment of CSK with the use of tacrolimus and DMSO causes the reduction in terms of inflammatory processes and neovascularisation, but in many cases does not inhibit the progress of the pigmentation.

Key words: dog, chronic superficial keratitis, tacrolimus, DMSO

Introduction

Canine chronic superficial keratitis (CSK) is an inflammatory ocular disease of autoimmune character which, if untreated, leads to blindness. Early symptoms allowing the diagnosis include reddening of the conjunctiva and the occurrence of a bilateral lesion in the inferior temporal quadrants starting in the region of the corneal limbus. The extension of blood vessels into the cornea, followed by the formation of the fibrous tissue and pigmentation begins in the inferior temporal quadrants. The fibrous tissue is initially thin, but as the disease progresses, the affected area of the cornea grows and the tissue can become

Correspondence to: I. Balicki, e-mail: balicki.ireneusz@gmail.com, tel.: +48 603 066 376

thicker, at times causing the corneal surface to become uneven. Additionally, in the central areas of the cornea, grey, focal, macular opacities can occur. In most cases, symptoms include swelling and depigmentation of the nictitating membrane margin. The inflammatory process typically affects both eyes, but the corneal lesions are usually not symmetrical (Chavkin et al. 1994, Balicki 2005, Balicki and Trbolova 2010).

It is considered that CSK is a complex autoimmune disorder and several additional genetic and environmental factors probably contribute to the etiology of the disease (Jokinen et al. 2011).

Pharmacological treatment of CSK is based on corticosteroids which can be administered as ophthalmic drops, ophthalmological ointment, or subconjunctival injections (Bedford 1979, Williams 1995, Clerk 1996, Balicki 2008). One of the most important medications in the treatment of CSK is cyclosporine, which can also be used in long-term therapy (Balicki 2005).

Efforts are constantly being made to find new therapeutic methods that would ensure the reduction of inflammatory process and pigmentation of the cornea. Studies have been conducted into the use of pimecrolimus in the treatment of CSK. The results indicate that in some clinical cases pimecrolimus can cause regression of the corneal vascularisation and pigmentation (Nell et al. 2005). Studies have also been conducted into the use of tacrolimus in CSK therapy (Balicki and Trbolova 2010). Tacrolimus is an antibiotic macrolide with immunosuppressant and antiinflammatory properties isolated from *Streptomyces tsukubaensis* (Kino at al. 1987). The medication acts on early activation of T lymphocytes, most likely preventing the transcription of T lymphocyte stimulation gene (IL-2, IL-3,IL-4,IFN-y,TNF-α, GM-CSF,*c-myc*) (Nghiem et al. 2001, Sakuma et al. 2001). Tacrolimus in the form of 0.02% ophthalmic drops has been administered in the treatment of keratoconjunctivitis sicca in dogs (Berdoulay et al. 2005). It has also been successfully used in the form of 0.03% ophthalmic drops in the treatment of blepharokeratoconjunctivitis, keratoconjunctivitis and chronic follicular conjunctivitis (Joseph 2005). Clinical and cytological conjunctival studies have also been conducted on patients treated with 0.03% tacrolimus in connection with blepharoconjunctivitis or keratoconjunctivitis, which indicated a reduction of conjunctival inflammatory cells' infiltration into the conjunctiva and lessening conjunctivitis (Virtanen 2006). It has been demonstrated that tacrolimus in the form of 0.02% ophthalmic drops can be administered in the treatment of chronic superficial keratitis in dogs. It causes the reduction of inflammatory process and neovascularisation of the cornea, but in certain cases does not inhibit the development of the pigmentation (Balicki and Trbolova 2010).

Dimethyl sulfoxide (DMSO) is a drug characterized by low toxicity and good tissue permeability, and facilitates the penetration of other substances through biological membranes. It also displays ant-inflammatory qualities, and analgesic, weak bacteriostatic and diuretic action as well as the ability to remove free radicals (Brayton 1986, Binnick et al. 1977, Del Maestro et al. 1989). Attempts have been made to employ DMSO in the treatment of ophthalmological diseases. Experimental studies demonstrated the beneficial action of DMSO in cases of chemical burning of the cornea in rabbits (Skrypuch et al. 1987). DMSO ophthalmic drops administered in concentrations of 50% and 60% accelerated the healing of corneal ulceration in rabbits (Toczołowski et al. 1992, Kawa 1997). A DMSO solution of vidarbine injected into the vitreous humor of rabbits proved effective in the treatment of viral infections (Yoshizumi et al. 1986). Similarly, a DMSO solution of ketoconazole injected into the vitreous humor of rabbits may be used in the treatment of fungal endophthalmitis (Yoshizumi and Banihashemi 1988). 30% DMSO ophthalmic drops were used in combination with 1% itraconazole (Ball et al. 1997) or with 0.2% flukonazole (Balicki et al. 2008) in the treatment of mycotic keratitis in horses. In the latter study, DMSO was also administered to horses suffering from defects of the anterior corneal epithelium, which resulted in curing 8 out of 10 clinical cases. In combination with other medicines such as phenylbutazone, atropine and itraconazole, DMSO was also used in the treatment of corneal ulceration caused by Listeria monocytogenes in mares (Sánchez et al. 2001). DMSO was used in the treatment of CSK and in a long-term CSK therapy (Balicki 2005). It was administered in combination with corticosteroids or cyclosporine (Balicki 2005, Balicki 2006). The study has revealed that the administration of topically combined 50% DMSO drops and dexamethasone is a more effective treatment of chronic superficial keratitis than the administration of dexamethasone alone (Balicki 2006). Clinical tests and examinations of corneal cells with the use of impression cytology did not indicate harmful effects of DMSO or DMSO combined with cyclosporine administered into the conjunctival sac over a period of 8 months (Balicki 2005).

The objective of this research was to assess the efficiency of chronic superficial keratitis treatment with the use of 0.02% tacrolimus combined with 50% DMSO.

Materials and Methods

The study was conducted on 16 dogs – 7 males and 9 females, aged 3 to 11 years, diagnosed with CSK. The patients underwent detailed ophthalmic examinations using slit-lamp biomicroscopy (Shin Nippon) as well a direct (Welch Allyn), indirect (Keeler) and panoptic ophthalmoscope – PanOptik (Welch Allyn) a tonometer – Tonopen (Reichert). Schimmer's tests were also performed (Eickemeyer).

The disease was treated with ophthalmic drops containing 0.02% tacrolimus and 50% DMSO, administered to the ocular surface three times a day. The drugs were formulated in 0.9% sodium chloride by a specialist in the field of ophthalmic pharmacy. Prior to the therapy and after 5 weeks, the following was determined: conjunctiva redness – lack (-), present (+); occurrence of ocular discharge; depigmentation of the third eyelid margin – present (+), absent (-); corneal area surface affected by the inflammatory process as well the occurrence of the pigmentation and corneal neovascularization. Where mucopurulent exudate was found in the conjunctival sac, the therapy was used in conjunction with 0.3% gentamicin drops administered three times a day for 14 days. Periodic photographic documentation was taken in all patients. The percentage of the corneal area involved in the inflammatory process, *i.e.* neovascularization, fibrous tissue, 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 (Figs. 1, 2). The corneal pigmentation was evaluated on the basis of its formation or regression, increased or decreased transparence rate, as well as a calculation of the number of corneas affected by the pigmentation prior to and 5 weeks after the treatment in all the patients. Blood vessel ingrowth into each corneal quadrant was also assessed and graded on the scale of 1 to 4 points.

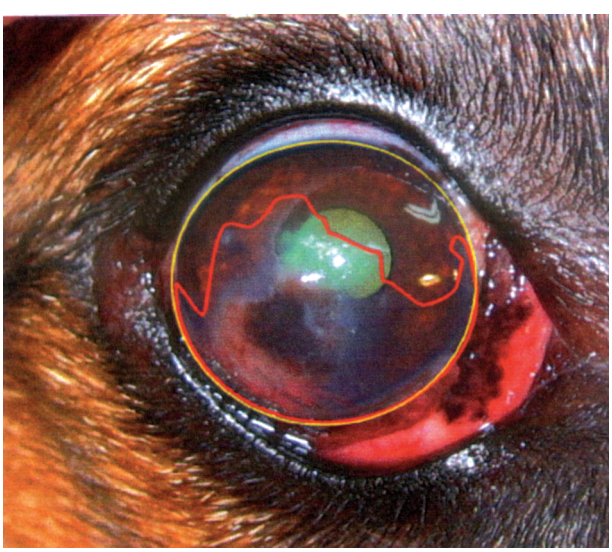

Fig. 1. Case 7: before treatment tacrolimus and DMSO – superficial macular opacities, pigmentation and neovascularization of the cornea. Yellow and red line enabled to calculate the percentage of the corneal area involved in the inflammatory process, with the use of IsoCalc.com's Get Area software for Corel DRAW 12

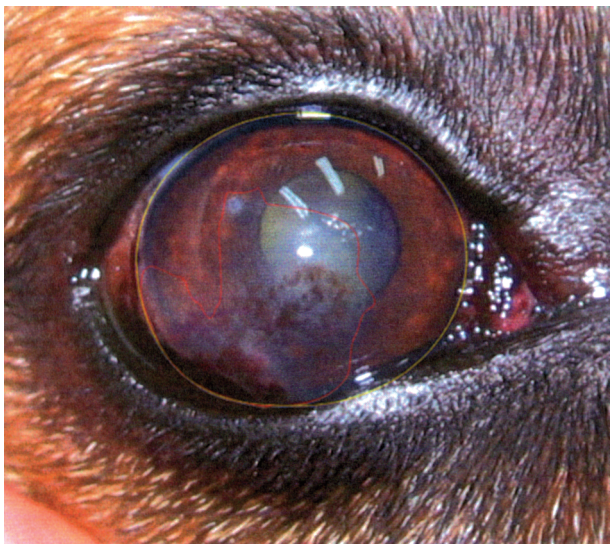

Fig. 2. Case 7 after a 5 week treatment with tacrolimus and DMSO – regression of corneal lesions.

The results were statistically analyzed. 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 methods in Statistica 9.0 (StatSoft). 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

Apart from CSK, no other ophthalmological disorders were observed in the dogs. Information obtained from the owners of the research group German shepherds indicated that in 6 dogs, DMSO ophthalmologic drops caused irritation of the eyeball, manifested by the animals through rubbing the affected area shortly after the administration. The irritation tended to subside within 5 minutes. The owners then reported that the dogs suffering from the above side-effects got accustomed to the eye drops within 2-3 weeks, after that period the symptoms of irritation would not occur.

Tacrolimus and DMSO treatment resulted in a reduction of the diagnosed conjunctival reddening.

Table 1. Responses to tacrolimus and DMSO therapy.

Case	Age	Sex	Eye	Depigmentation of the third eyelid margin		Corneal neovascularization (number of quadrant)		Corneal area affected (%)		Corneal pigmentation			
											after treatment		
				before treatment	after treatment	before treatment	after treatment	before treatment	after treatment	befor treatment	Decrease	Develop-ment	decrease density
1	10	♂	OD	D	R	3	0	67	49	+	+		+
			OS	D	R	4	2	78	58	+		+	+
2	7	♀	OD	D	R	3	2	52	31	+	+	+(?)	+
			OS	D	R	4	3	81	51	+	+		+
3	8	♂	OD	D	R	3	3	68	45	+		+(?)	
			OS	D	R	3	3	54	26	+		+(?)	
4	11	♂	OD	D	R	3	3	78	48	+		+(?)	
			OS	D	R	3	1	30	18	+		+(?)	
5	5	♀	OD	D	R	4	2	87	45	+		+	
			OS	D	R	4	2	95	49	+	+		+
6	3,5	♂	OD	D	R	4	1	78	31	+		+	
			OS	D	R	4	2	73	47	+		+	+
7	4,5	♀	OD	D	D	2	1	63	41	+	+	+(?)	+
			OS	D	D	1	1	46	10	+	+		+
8	6	♀	OD	D	R	2	1	24	18	+	+		+
			OS	D	R	1	1	10	12	+		+	
9	7	♀	OD	D	R	4	2	100	45	+		+	
			OS	D	R	4	2	98	51	+	+	+	
10	5	♀	OD	D	R	2	2	37	20	+		+	
			OS	D	R	1	1	13	12	+		+(?)	
11	3	♂	OD	D	R	1	0	15	8	+		+(?)	
			OS	D	R	1	1	13	7	+		+	
12	5	♀	OD	D	R	4	2	88	38	+		+	+
			OS	D	R	4	2	71	47	+		+	+
13	4	♂	OD	D	R	3	1	79	63	+	+	+	+
			OS	D	R	2	0	39	29	+	+	+	+
14	6	♀	OD	D	R	4	2	94	80	+	+	+(?)	+
			OS	D	R	4	2	96	82	+		+(?)	
15	5	♀	OD	D	R	4	3	92	52	+	+		
			OS	D	R	4	1	53	19	+		+	
16	5	♂	OD	D	R	4	4	91	88	+	+	+	
			OS	D	R	4	4	92	100	+	+	+	

Table 2. Depigmentation of the third eyelid margin.

Before treatment	After treatment	
Number of the third eyelid with depigmentation	Repigmentation of the third eyelid margin	Depigmentation of the third eyelid margin
32	30	2

Prior to the treatment, the presence of purulent-mucus exudation was observed in the medial canthus of 13 patients. The exudation would disappeared after 7 to 14 days of administering gentamicin.

Treatment effects in particular patients are presented in Table 1. The depigmentation of the nictitating membrane margin was diagnosed in all patients prior to the treatment. As a result of the treatment, the repigmentation of the left and right nictitating membrane margins (Figs. 3, 4) was observed in 15 patients (Tables 1, 2).

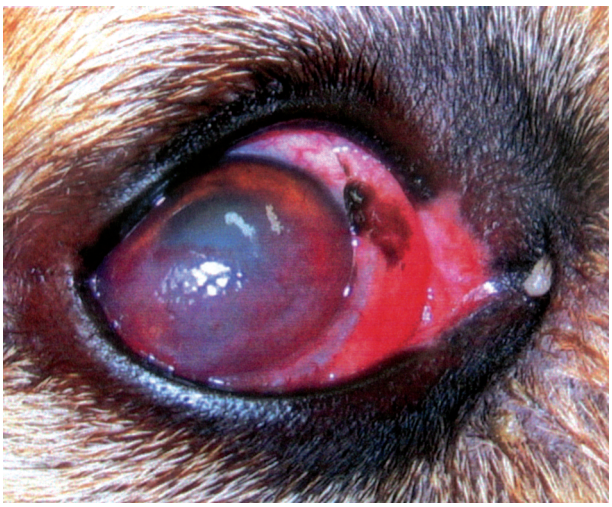

Fig. 3. Case 6: neovascularization, fibrous tissue of the ventral cornea quadrants and depigmentation of the free margin of the nictitans membrane.

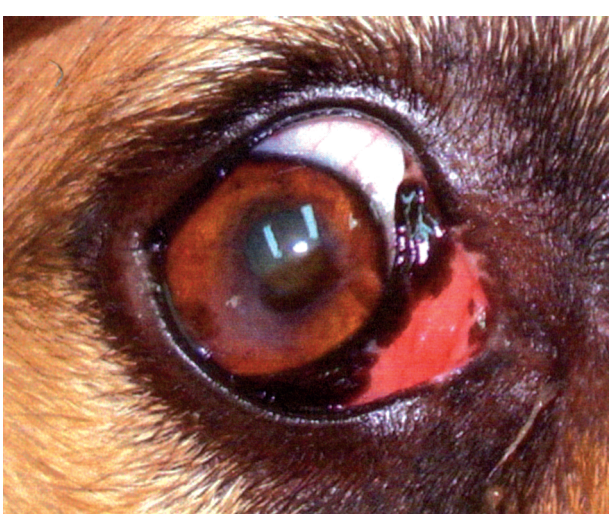

Fig. 4. Case 6: the disappearance of the fibrous tissue and corneal neovascularization, repigmentation of the third eyelid margin; due to the presence of the extensive fibrous tissue before treatment, the assessment of the pigmentation was not possible.

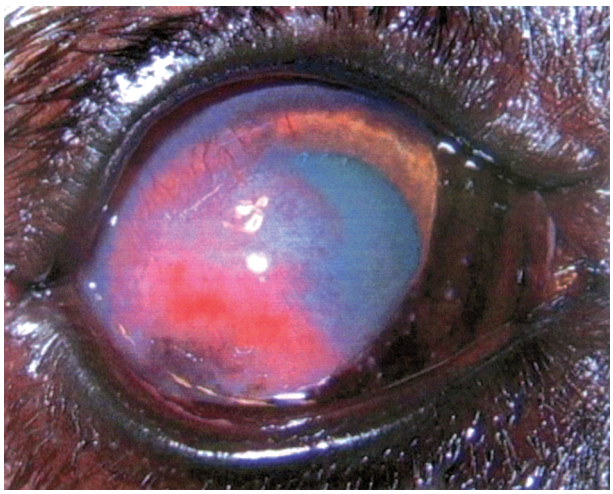

Fig. 5. Case 4: neovascularization, pigmentation and fibrous tissue of the temporal cornea quadrants.

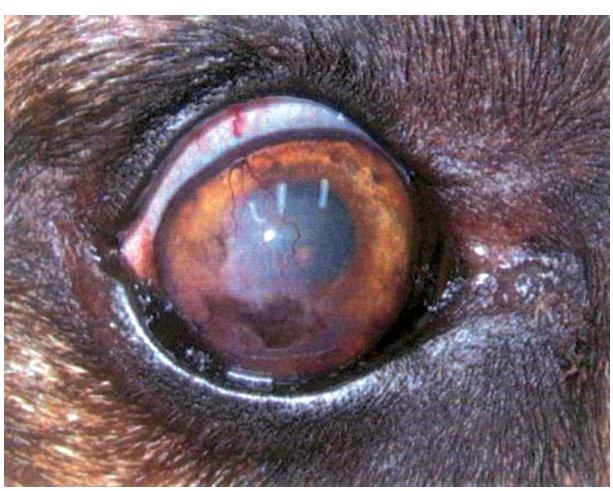

Fig. 6. Case 4: the disappearance of the fibrous tissue and corneal neovascularization, due to the presence of the extensive fibrous tissue before treatment, the assessment of the pigmentation was not possible.

The treatment led to a reduction in the neovascularisation of the cornea (Tables 1, 3); (Figs. 5, 6). Beforehand, neovascularisation extended over a total of 50 and 48 quadrants of the right and left corneas

Table 3. The area of the affected cornea and corneal quadrants with neovascularization before and after a 5-week treatment (x ± SD).

	Before treatment		After treatment	
	right cornea	left cornea	right cornea	left cornea
Corneal area affected (%)	69.6 ± 25.6	58.9 ± 31.2	43.9** ± 21.0	38.6 ± 27.0
Corneal quadrants with neovascularization	3.12 ± 0.95	3.0 ± 1.31	1.81** ± 1.11	1.75** ± 1.0
Number of all quadrants with neovascularization	50	48	29***	28***

Explanations: statistically significant differences ** P ≤ 0.01; *** P ≤0.001

Table 4. Corneal pigmentation.

	Before treatment	After treatment		
	number of corneas affected	decrease	development	decrease density
Corneal pigmentation	32	14	16; 10(?)	17

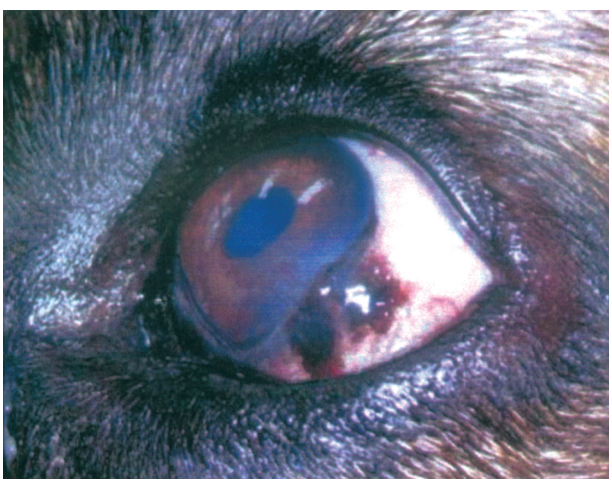

Fig. 7. Case 8: inflammatory process of the ventrolateral cornea quadrants.

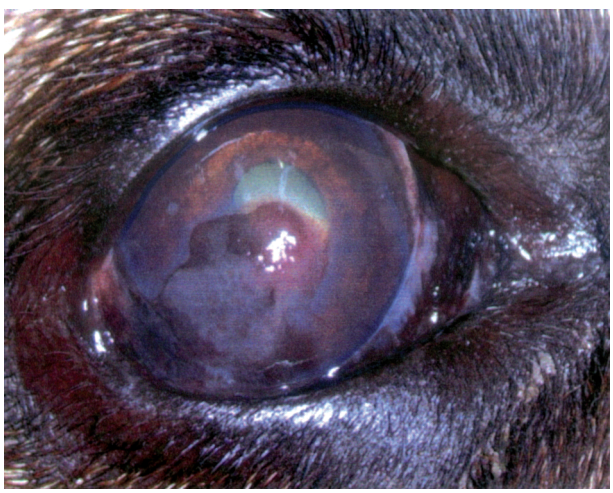

Fig. 9. Case 8: the pigmentation and corneal neovascularization.

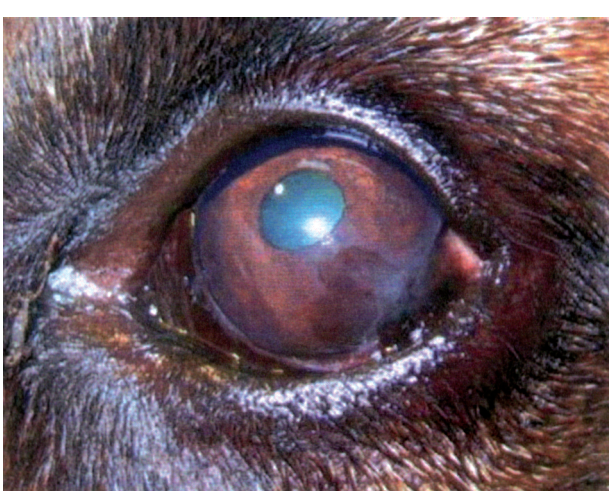

Fig. 8. Case 8: the development of the pigmentation.

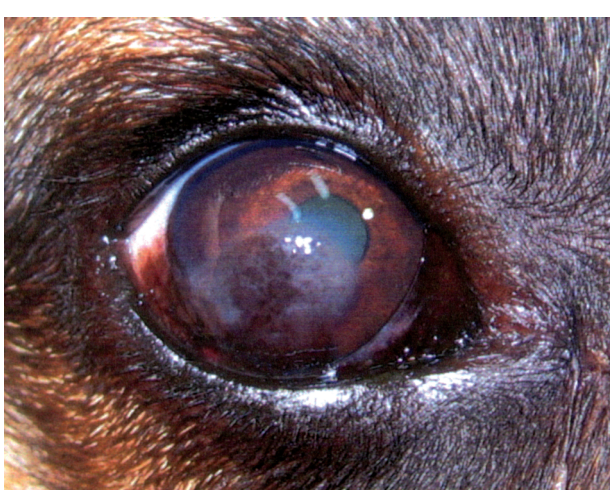

Fig. 10. Case 8: The reduction of the pigmentation and neovascularization.

respectively. After the treatment, it was statistically significantly reduced to 29 ($p \leq 0.001$) and 28 ($p \leq 0.001$) quadrants of the right and left corneas, respectively. The mean number of the right corneal quadrants affected by neovascualrisation was statistically significantly reduced from 3.12 to 1.81 ($p \leq 0.01$), while for the left corneas, from 3 to 1.75 ($p \leq 0.01$).

It was observed that the CSK therapy with the use of tacrolimus and DMSO resulted in reduction of the inflammatory process of the cornea in all the patients (Tables 1, 3); (Figs. 5, 6). The mean corneal area affected by the disease process was statistically significantly reduced from 69.6% to 43.9% ($p \leq 0.01$) – in case of the right corneas, and from 59.9% to 38.6 in case of the left corneas. In 5 patients (No. 2, 4, 5, 6, and 9) the thick fibrous tissue was observed. Tacrolimus and DMSO caused a reduction of the size and thickness of the fibrous tissue. Before the treatment, the corneal pigmentation was observed in all the patients. Of the 32 corneas affected, the pigmentation was reduced in 14 corneas of 10 dogs (Tables 1, 4). In case of 5 corneas (dog No. 9, 13, 16), areas of reducing and growing pigmentation were observed simultaneously. In 7 dogs (No. 1, 5, 8, 10, 13, 15, 16) the

pigmentation was observed to grow in one cornea while simultaneously increasing in the other (Figs.7, 8, 9, 10). The increased pigmentation was observed on the surface of 16 corneas in 11 patients. In case of 10 corneas, due to the presence of the extensive fibrous tissue, the assessment of the pigmentation was not possible (Figs. 5, 6). The treatment resulted in an observable improvement of transparence in 17 corneas.

Discussion

It is believed that in the first stage of CSK treatment, ophthalmologic medicines should be administered in frequent dosages (Stadec et al. 2007, Balicki 2008, Gliger et al. 2009), which is often burdensome for the dog's owner and stressful for the dog itself. The animal's stress results from both the frequent administration of medicine and the temporary irritation characteristically caused by many ophthalmologic medications, as demonstrated in author's own research focused on the irritant effects of DMSO. In the present study, good therapeutic effects were observed when administering tacrolimus and DMSO only three times a day, which helped to reduce the patient's discomfort. Earlier clinical studies indicated the probability of lens lesions occurring as a side effect of oral administration of DMSO. Lens lesions in dogs after a long-term (over 9 week) administration of DMSO were first reported by Rubin and Barnett (1967). They observed opacities in the nuclei of lenses which lead to disorders in terms of eye refraction. Similar adverse effects connected with the dosage of DMSO have been described by a number of other researchers (Noel et al. 1975, Rossa and Kluxen 1988). Lens opacities have not been reported when administering DMSO in the form of drops applied to the conjunctival sac in humans (Gordon 1967), rabbits (Toczołowski et al. 1992, Kawa 1997), horses (Ball et al. 1997, Sanchez et al. 2001), or dogs (Balicki 2005, Balicki 2006). Administration of ketoconazole and DMSO into the vitreous humor has also been reported not to have an adverse effect on anatomical structures of the eye (Yoshizumi and Banihashemi 1988). In author's own research, no side effects of DMSO were observed, apart from short-term irritation reported in some patients. It is believed that tacrolimus does not display irritant effects and is generally well tolerated by patients. No complications were reported in relation to applying ointment containing 0.03% tacrolimus in the treatment of allergic conjunctivitis in humans (Attas-Fox 2008). In the treatment of giant papillary conjunctivitis in humans, no side effects were reported after administering 0.03% tacrolimus ointment for a period of 3 months (Kymionis et al. 2008). Earlier studies did not reveal adverse effects of tacrolimus administered three times a day for a period of 5 weeks (Balicki and Trbolova 2010). The present study has confirmed that tacrolimus is tolerated well by patients. None of the treated dogs were observed to suffer from irritation in the form of a reddening or itching of the conjunctiva. Tacrolimus is used in the treatment of a number of immunological conditions but the scope of its application in dogs has never been determined. Therefore, due to the possibility of unforeseen side effects, it must be administered with due discretion (Mags 2008). For that reason, in the course of the study discussed tacrolimus was administered only three times a day for a period of 5 months. It is believed that the first month of CSK treatment provides ample information on medications' effectiveness and allows an informed decision concerning adequate therapy in the long-term (Gilger et al. 2007).

The presence of significant purulent-mucus exudation in 81% of the patients should be highlighted. Most literature does not report the presence of bacterial infections of the conjunctival sac in cases of CSK. Research conducted previously by the author revealed the presence of purulent-mucus exudation in the conjunctival sac of many dogs suffering from CSK (Balicki 2005, Balicki and Trbolova 2010). It should be assumed that the conditions stem from secondary bacterial infections, but further research is needed to establish the actual causes of such high incidence of infections in CSK cases.

All pathological lesions observed in the cornea are preceded by the neovascularisation. Therefore, the disappearance of the corneal neovascularisation is a good prognostic symptom signifying the recession of the disease process. Tacrolimus and DMSO caused a statistical reduction of the corneal neovascularisation ($p \leq 0.001$). Of the 98 corneal quadrants affected by the neovascularisation prior to the treatment, the condition was diagnosed in only 57 quadrants after the therapy – which means that the number of quadrants affected by the neovascularisation was reduced by 42% after 5 weeks of the treatment. Nell et al. (2005) demonstrated that in certain cases the regression of the neovascularisation could be observed as early as after 2 weeks of administering pimecrolimus, and full disappearance after 11 weeks. In author's own research complete disappearance of the neovascularisation in 4 corneas was observed after a 5 week therapy with tacrolimus and DMSO. It should be observed that the treatment of CSK with tecrolimus and DMSO leads to the regression of the corneal neovascularisation in most cases, while in some it results in its complete disappearance.

Due to the possibility of the corneal neovascularization and fibrous tissue being covered by the pigmentation, in some cases the presence of pigmentation under thick fibrous tissue was accounted for by the author in the calculated percentage of the total corneal surface affected by the entire disease process, that is the neovascularization, fibrous tissue, superficial macular opacities and pigmentation. As a result of the tacrolimus and DMSO treatment, the mean area of the cornea affected by the disease process was statistically significantly reduced by 26% ($p \leq 0.01$) in case of the right corneas, and by 20% in case of the left corneas. In previously conducted research, the author demonstrated that CKS treatment with the use of 0.02% tacrolimus drops resulted in the reduction of the corneal surface affected by the disease process by 25% and 9%, respectively in the right and left corneas (Balicki and Trbolova 2010). It should therefore be concluded that combining tacrolimus with DMSO leads to only a slight improvement of the treatment results. However, one should also consider the fact that in the research where only tacrolimus was used in the CSK treatment, the median of the corneal area affected by the disease was 58% and 46% in the right and left corneas, respectively. The advancement of the disease process at the onset of the treatment was therefore lesser than in the study in which dogs were treated with tacrolimus and DMSO simultaneously, in which case the relative mean of the corneal area affected by the disease was approximately 70% and 59% for the right and left corneas, respectively. Stimulating regression of the disease process in such an advanced stage is, undoubtedly, more difficult. It should be stressed that tacrolimus combined with DMSO not only ensured the regression of the disease but also caused the reduction of the inflammatory fibrovascular infiltration. Tacrolimus and DMSO reduced its thickness, as was confirmed in all 4 patients diagnosed with a thick fibrous tissue prior to the treatment.

The present study also assessed the occurrence or disappearance as well as increase or decrease in the transparence of the visible pigmentation. In many cases of CSK it is difficult to determine the area of the cornea affected by the pigmentation. It can cover an inflammatory infiltration, in some cases it can also be impossible to precisely determine the occurrence of the pigmentation due to its location under a thick fibrous tissue. Due to the above, in the course of this study, the reduction or growth of the pigmentation could not be precisely determined in case of 10 corneas. The most difficult element of a CSK therapy is the inhibiting the development of the pigmentation and ensuring its regression. In the course of the CSK treatment with the use of tacrolimus without DMSO, a decrease of the extent of the pigmentation was observed in 13 of 27 corneas affected by the pigmentation prior to the therapy, while at the same time the pigmentation grew in 8 cases (Balicki and Trbolova 2010). Before the start of the therapy using tacrolimus in combination with DMSO, 32 corneas were diagnosed with the pigmentation. After the treatment, the pigmentation decrease was observed in 14 corneas, while increase in 16. It should therefore be concluded that the addition of DMSO did not result in the reduction of the pigmentation. The effect of medication on the pigmentation was not clear – in some dogs the pigmentation was observed to grow in one cornea while simultaneously increasing in the other. Furthermore, in 5 corneas some areas of the pigmentation would shrink while others expanded. In advanced stages of the disease, where the extensive corneal pigmentation was located in the superficial layers of the stroma, regression of the pigmentation is often impossible. In such cases long-term inhibition of the further development of the pigmentation should be considered a determinant of successful treatment. In a number of clinical cases, tacrolimus and DMSO were observed not to inhibit the development of the pigmentation but rather to increase its transparence. Similar results were reported by Williams et al. in 1995. They demonstrated that both cyclosporine and dexamethasone, when applied in the treatment of CSK, may not reduce the area of the cornea affected by the pigmentation but will increase its transparence.

In a majority of CSK cases swelling and depigmentation of the nictitating membrane margin was reported. Bigelbach (1994) observed complete repigmentation of the nictitating membrane after long-term treatment. In author's own research it was demonstrated that the application of tacrolimus and DMSO in CSK treatment in most cases results in the repigmentation of the nictitating membrane. Of the 32 nictitating membranes affected by the depigmentation, the treatment caused the repigmentation of 30, i.e. 94%. When treated with tacrolimus alone, the repigmentation was also observed, however of 28 nictitating membranes affected, the repigmentation was observed in 22, which constituted 79% (Balicki and Trbolova 2010). The above indicates a higher probability of the nictitating membrane repigmentation in dogs treated for CSK with a combination of DMSO and tacrolimus.

Conclusion

The present studes has demonstrated that 0.02% tacrolimus and 50% DMSO may be applied in the treatment of chronic superficial kertatitis in dogs. The therapy results in reducing the inflammation and

neovascularisation but in many cases does not inhibit the spread of the pigmentation.

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