CLINICAL EVALUATION OF TACROLIMUS EYE DROPS FOR CHRONIC SUPERFICIAL KERATITIS TREATMENT IN DOGS

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Received for publication November 27, 2009

Abstract

The aim of this study was to assess the efficacy of 0.02% tacrolimus ophthalmic drops application for chronic superficial keratitis (CSK) treatment in dogs. The studies included 14 German Shepherd dogs – eight males and six females, aged 2-10 years affected with CSK. The drops were administered to the ocular surface three times a day. Prior to the treatment onset, and after the 5 week medical therapy, an estimation of a conjunctiva redness, ocular discharge, depigmentation of the third eyelid, and blood vessel ingrowth in each corneal quadrant and corneal pigmentation was conducted. The photo images with calibrated grid enabled to calculate the percentage of corneal area surface affected by inflammatory process. Tacrolimus did not exert any irritant effects throughout the treatment. The therapy has led to the decrease in corneal inflammatory infiltrate and blood vessel ingrowth in all the patients. Median corneal area surface affected by the condition showed a statistically significant decrease from 46% to 27% (P< 0.01) in case of the left corneas and from 58% to 33% for the right ones. Out of 27 corneas affected by pigmentation, 13 corneas in eight patients exhibited decreased pigmentation. The increased pigmentation was observed on eight corneas in five patients. The studies proved that 0.02% ophthalmic drops of tacrolimus have been effective topically in CSK therapy. A treatment response was observed by reduced granulation tissue and corneal neovascularisation, still in some cases tacrolimus failed to inhibit the pigmentation formation.

Key words: dog, chronic superficial keratitis, tacrolimus, therapy.

Chronic superficial keratitis (CSK) is a progressive condition with a main clinical manifestation of a fibrovascular infiltration commencing at the lower temporal corneal quadrants, combined with corneal neovascularisation and eventually followed by pigmentation. The initial clinical appearance of the disease includes a slight inflammatory infiltrate in the region of the limbus of lower temporal corneal quadrant. The abnormal ingrowth of blood vessels into the cornea and an inflammatory infiltration develops in the form of a pink fibrovascular tissue (12, 17, 23). Initially, it is thin but as the disease progresses, the infiltrate gets thicker and occasionally, the corneal surface becomes uneven. In addition, centrally located corneal focal macular opacities of grey colour are detected. Most dogs manifest the typical CSK lesions in the cornea as well as oedema and depigmentation of the third eyelid margin. The inflammatory processes are bilateral but the corneal changes are usually asymmetrical. Some dogs experience loss of the corneal transparency in short time, while others manifest slow progression of the condition with maintained good vision acuity (8). CSK is thought to be immune mediated corneal disease (26). The disease may develop for a few months or even some years and in severe cases may cause blindness a year after the symptom onset (9).

The main therapy for the CSK condition includes administration of topical corticosteroids in the form of ophthalmic drops or ointment directly to the conjunctival sack (3, 5, 10, 17, 25). They may be also applied as subconjunctival injections. Cyclosporine used alone or in conjunction with topical corticosteroids is effective in cases of CSK (3, 7, 25). Williams et al. (25) compared the medical outcomes of the CSK therapeutic processes with 0.2% cyclosporine and 0.1% dexamethasone. They found that cyclosporine application implicated the disappearance of blood vessels and corneal infiltration in a similar manner to dexamethasone action (25). There were also some clinical studies on the use of ophthalmic drop of dimethyl sulfoxide (DMSO) for CSK condition therapy (3, 4). Its combined application with dexamethasone proved to be more efficient than dexamethasone alone (40). Recently, the use of pimecrolimus in CSK therapy became an object of researches. They revealed that in some clinical cases pimecrolimus caused the regression of both, blood vessel ingrowing and corneal pigmentation (19). However, with the current therapies,
chronic superficial keratitis can usually be controlled by medical or surgical therapy, by it cannot be cured (12).

Tacrolimus is a macrolide antibiotic with immunosuppressant and anti-inflammatory properties isolated from *Streptomyces tsukubaensis* (14). The medication acts on early activation of T lymphocytes, most likely preventing the transcription of T lymphocyte stimulation genes (IL-2, IL-3, IL-4, IFN-γ, TNF-α, GM-CSF, c-myc) (20, 21). Tacrolimus is commonly employed in human and animal therapy, predominantly in transplantology and dermatology but its use in ophthalmology is still limited (11, 13, 15, 18, 24). Ophthalmic drops of 0.02% tacrolimus were used to treat canine keratoconjunctivitis sicca (6). Topical 0.03% tacrolimus ointment appeared to be a well tolerated and potent drug for human allergic conjunctivitis treatment (2). The positive effects of 0.03% tacrolimus employment in the therapeutic process of blepharokeratoconjunctivitis, keratoconjunctivitis, and chronic follicular conjunctivitis were reported (13). Conjunctival cytology, as well as clinical examination of the patients, who underwent the 0.03% tacrolimus therapy towards blepharoconjunctivitis or keratoconjunctivitis were also performed. The studies have revealed the reduced infiltration of inflammatory cells in the conjunctiva and improvement in conjunctivitis (24).

The objective of the presented research has been to assess the efficacy of 0.02% tacrolimus ophthalmic drops application for chronic superficial keratitis treatment in dogs.

**Material and Methods**

The studies included 14 German Shepherd dogs – eight males and six females aged 2-10 years, affected with *keratitis superficialis chronica*. The patients underwent the detailed ophthalmic examinations using a slit lamp, as well as the indirect and direct ophthalmoscopy. The patients were treated with eye drops of 0.02% tacrolimus in 0.9% sodium chloride formulated by a specialist in the ophthalmic pharmacy field. The drug was administered three times a day to the ocular surface. Prior to the therapy, and 5 weeks after it, the following features were determined: conjunctiva redness - lack (-), present (+); occurrence of ocular discharge; depigmentation of the third eyelid margin - present (+), absent (-); corneal area surface affected by inflammatory process, as well as the occurrence of pigmentation and corneal neovascularisation. When mucopurulent exudate was observed, the tacrolimus therapy was used in conjunction with 0.3% gentamicin ophthalmic drops administered three times a day for 14 d. The repeated photographic documentation of all patients was taken. The calibrated grid was placed on photographs to calculate a percentage of the corneal area involved in inflammatory process, *i.e.* neovascularisation, granulation tissue, superficial macular opacities, and pigmentation (Fig. 1). The corneal pigmentation was evaluated on the basis of its formation or regression, increased or decreased transparency rate, and calculation of a number of corneas affected by pigmentation prior to, and 5 weeks post the treatment in all the patients. Calculation of the blood vessel ingrowth into each corneal quadrant – graded from 1 to 4 grade points was also conducted.

The research results were analysed statistically. The Kolmogorov-Smirnov test showed a non-normal distribution of data, therefore the Mann-Whitney test was used to compare the parameters before and after the 5-week treatment. The *χ*-square test was used to compare totals of corneal quadrants involved in neovascularisation.

**Results**

Tacrolimus application reduced the conjunctiva redness. None of the patients displayed irritated conjunctivas as the effect of the medication action, manifested by increasingly red and itching eyes. Before and onset of the therapy, three patients were diagnosed as having mucopurulent exudate visible in the medial angle of the lid. The exudate gradually disappeared on 10 to 14 d of the gentamicin therapy.

The overall treatment outcomes obtained for each patient are summarised in Table 1. Tacrolimus therapy was associated with reduced neovascularisation of the cornea (Figs 1 and 2).

**Fig. 1.** Case 13. Before tacrolimus treatment – superficial macular opacities, pigmentation, and neovascularisation of the temporal cornea quadrants (cornea with calibrated grid).

**Fig. 2.** Case 13. After a 5 week treatment with tacrolimus – regression of corneal neovascularisation.
| Case | Age | Sex | Eye | Depigmentation of the third eyelid margin | Corneal neovascularisation (number of quadrant) | Corneal area affected (%) | Corneal pigmentation | Before treatment | After treatment | Before treatment | After treatment | Before treatment | Decrease | Development | Decreased density |
|------|-----|-----|-----|------------------------------------------|-----------------------------------------------|--------------------------|----------------------|-------------------|----------------|----------------|----------------|----------------|----------------|-----------|-------------|------------------|
| 1    | 3   | ♂   | OD  | D  | D  | 4 | 4 | 68 | 52 | + | + | + | + | + | + | + | + | + | + | + |
| 2    | 10  | ♀   | OD  | D  | D  | 4 | 2 | 85 | 50 | + | + | + | + | + | + | + | + | + | + | + |
| 3    | 9   | ♂   | OD  | D  | R  | 4 | 2 | 100 | 82 | + | + | + | + | + | + | + | + | + | + | + |
| 4    | 4   | ♀   | OD  | D  | R  | 1 | 1 | 20 | 14 | + | + | + | + | + | + | + | + | + | + | + |
| 5    | 3   | ♀   | OD  | D  | R  | 1 | 1 | 24 | 20 | + | + | + | + | + | + | + | + | + | + | + |
| 6    | 3   | ♂   | OD  | D  | R  | 1 | 1 | 17 | 10 | + | + | + | + | + | + | + | + | + | + | + |
| 7    | 9   | ♀   | OD  | D  | R  | 3 | 1 | 47 | 30 | + | + | + | + | + | + | + | + | + | + | + |
| 8    | 6   | ♂   | OD  | D  | R  | 4 | 3 | 78 | 62 | + | + | + | + | + | + | + | + | + | + | + |
| 9    | 7   | ♀   | OD  | D  | D  | 2 | 1 | 38 | 7 | + | + | + | + | + | + | + | + | + | + | + |
| 10   | 4   | ♂   | OD  | D  | R  | 2 | 2 | 38 | 33 | + | + | + | + | + | + | + | + | + | + | + |
| 11   | 6   | ♂   | OD  | D  | R  | 4 | 1 | 71 | 32 | + | + | + | + | + | + | + | + | + | + | + |
| 12   | 4   | ♂   | OD  | D  | R  | 4 | 2 | 78 | 60 | + | + | + | + | + | + | + | + | + | + | + |
| 13   | 7   | ♀   | OD  | D  | R  | 4 | 3 | 76 | 48 | + | + | + | + | + | + | + | + | + | + | + |
| 14   | 8   | ♂   | OD  | D  | R  | 3 | 1 | 48 | 32 | + | + | + | + | + | + | + | + | + | + | + |

Explanation: D- depigmentation, R- repigmentation, (?) difficult to evaluated.
### Table 2
Area of affected cornea and median number of quadrants with neovascularisation before and after a 5-week treatment

<table>
<thead>
<tr>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right cornea</td>
</tr>
<tr>
<td><strong>Median of corneal area affected (%) (min-max)</strong></td>
<td>58 (100-17)</td>
</tr>
<tr>
<td><strong>Median of quadrants with neovascularisation (min-max)</strong></td>
<td>3.5 (4-1)</td>
</tr>
<tr>
<td><strong>Number of all quadrants with neovascularisation</strong></td>
<td>41</td>
</tr>
</tbody>
</table>

(min-max) – minimal and maximal values, * P<0.05; ** P<0.01.

### Table 3
Corneal pigmentation

<table>
<thead>
<tr>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corneal pigmentation</td>
</tr>
<tr>
<td><strong>Number of corneas affected</strong></td>
<td>27</td>
</tr>
<tr>
<td><strong>Decrease</strong></td>
<td>13; 1(?)</td>
</tr>
<tr>
<td><strong>Development</strong></td>
<td>8; 7(?)</td>
</tr>
<tr>
<td><strong>Decreased density</strong></td>
<td>20</td>
</tr>
</tbody>
</table>

Fig. 3. Case 10. The ventral quadrants covered by thick granulation tissue.

Fig. 4. Case 10. The complete disappearance of granulation tissue, remaining of corneal neovascularisation. It was hard to estimate the progression or regression of pigmentation because of thick granulation tissue before treatment.
Fig. 5. Case 3. The whole cornea covered by superficial macular opacities, neovascularisation and pigmentation.

Fig. 6. Case 3. A decrease in superficial macular opacities and pigmentation.

Fig. 7. Case 7: the pigmentation and corneal neovascularisation with grayish granulation tissue.

Fig. 8. Case 7: disappearance of neovascularisation and granulation tissue, the increase and decrease of pigmentation on the same cornea after a 5 week treatment with tacrolimus.

Fig. 9. Case 6. Neovascularisation of the ventrotemporal cornea quadrant and depigmentation of the free margin of nictitans membrane.

Fig. 10. Case 6. The disappearance of corneal neovascularisation. The repigmentation of the third eyelid margin and progression of corneal pigmentation.
Before the therapy, a total of 71 corneal quadrants were involved in neovascularisation, whereas their number after the treatment was 39. A median right corneal quadrants with neovascularisation was reported to show a statistically significant decrease from 3.5 to 1.5 while for the left corneas from 2 to 1.

It was found that tacrolimus therapy provided benefits to all the patients by reducing of corneal area surface affected by inflammatory process (Table 2). In addition, this medication contributed to the decrease in granulation tissue thickness (Figs 3 and 4). Median corneal area surface affected by the condition showed a statistically significant decrease from 46% to 27% (P<0.01) in case of the left corneas and from 58% to 33% for the right ones. The occurrence of pigmentation was recognised in all of the patients before the therapy but in one of them, the pigmentation involved only one cornea. Out of 27 corneas affected by pigmentation, 13 corneas in eight patients exhibited decreased pigmentation (Table 3; Figs 5 and 6), whereas increased pigmentation was observed on eight corneas in five patients. The increase and decrease in pigmentation on the same cornea was noted in patient No. 7 (Figs 7 and 8). It was not possible to accurately assess a decrease or increase in pigmentation on eight corneas because prior to the treatment, and at the beginning, a thick inflammatory infiltration was found in the sites where later pigmentation appeared (Figs 3 and 4). The tacrolimus therapy has decreased the density of pigmentation in 20 corneas.

Before the therapy, the depigmentation of the third eyelid margin was diagnosed in 14 patients. The outcome of the treatment was repigmentation of the third eyelid margin in 11 patients and persistent depigmentation in three dogs (Table 1; Figs 9 and 10).

**Discussion**

Corticosteroids have been the mainstay in CSK therapy. The authors indicate that the drugs are to be used at high frequency in the first period of therapeutic process (12, 22). It is believed that 1% prednisolone should be applied every 4 h for the first 7-10 d (17). The presented study has revealed that the therapeutic effects were obtained when tacrolimus was administered only three times a day for 5 weeks.

Tacrolimus as an immunosuppressive agent has proven to be a useful tool for the treatment of numerous immune-mediated diseases, but its therapeutic range for dogs has not been defined. Therefore, tacrolimus therapy requires special precautions due to potential adverse events that might develop (16). Having that in mind, this medication was used three times a day for only 5 weeks. The studies on its level in blood serum after 0.03% tacrolimus topical application twice a day did not show any significant systemic absorption after the 2-week application period (1).

Topical cyclosporine therapy led to side effects in some patients, including conjunctival irritation reactions (7, 25). However, the treatment of human giant papillary conjunctivitis did not produce any adverse events associated with topical 0.03% tacrolimus ointment applied for 3 months (15). Similarly, no common treatment-related side effects associated with 0.03% ointment of tacrolimus applied for human allergic conjunctivitis therapy were reported (2). The studies have confirmed good tolerability of the medication. The treated dogs did not display any signs of irritated eyes, *i.e.* conjunctiva redness or itching eyes as a response to 0.02% ophthalmic ointment of tacrolimus.

As for the CSK cases, it remains challenging to define the corneal surface area involved in granulation tissue or pigmentation. The pigmentation may cover the granulation tissue located underneath, or in some cases it is impossible to accurately localise pigmentation as it may develop under a thick granulation tissue. The examinations indicated the occurrence of the pigmentation after regression of granulation tissue. It is difficult to state if it became developed or regressed as it was not visible before the therapy commencement. Therefore, the authors estimate the percentage of corneal surface area affected by the disease, *i.e.* neovascularisation, granulation tissue, superficial macular opacities, and pigmentation. Additionally, the evaluation of the development or regression, and increase or decrease in the transparency of visible pigmentation was performed.

Regarding the CSK progression, the blood vessel ingrowing into the cornea is preceded by the development of granulation tissue. Owing to this fact, corneal neovascularisation regression appears to be a promising sign of the disease remission. The conducted studies have revealed a statistically significant reduction of the corneal neovascularisation. A number of quadrants under corneal neovascularisation declined by 45% as a therapeutic outcome of the 5-week therapy. Nell et al. (19) found that in some cases the corneal neovascularisation regressed as early as 2 weeks after pimecrolimus therapy and completely disappeared after 11 weeks. Pimecrolimus application caused a decrease in corneal opacity, vascularisation, and pigmentation. The presented study has shown that as for the right corneas, median corneal surface area involved in the condition decreased by 44.8%, and by 34.7% for the left corneas. Balicki (3) carried out the studies in the same manner and found that 0.5% prednisolone applied for the CSK treatment has induced a decrease in the affected corneal surface area by 35% for the right corneas, and 41% for the left corneas. Importantly, in this study, tacrolimus treatment resulted not only in a considerable disease regression but additionally, in the cases where a thick granulation tissue was formed, it effectively reduced its thickness.

The most challenging element of the CSK treatment is to suppress the pigmentation development and cause its regression. Progressing remission of corneal pigmentation was observed as a therapeutic effect of the pimecrolimus application (19). The study showed a reduced corneal pigmentation on 13 corneas in eight patients but pigmentation development was observed on eight cornea surface areas in five patients. It was found that pigmentation progressed despite a
decrease in the granulation tissue. Interestingly, in many clinical cases tacrolimus failed to suppress pigmentation production but increased its transparency. These findings are consistent with other results reported by Williams et al. (25). The authors found that when the dogs affected by CSK were treated with both cyclosporine and dexamethasone, the density of pigmentation was reduced even when a decreased response rate of the pigmentation-affected area of the cornea was not observed. In the severe cases, when the cornea has undergone extensive pigmentation localized in its superficial layers of the stroma, its regression is frequently impossible. Then, the inhibition of pigmentation formation and decrease in its density may be considered as a positive therapeutic outcome.

Besides, the evaluation of the corneal pigmentation in some CSK clinical cases is not easy. In the presented investigations, the pigmentation occurred in the site where a thick granulation tissue regressed. It was hard to confirm its presence and extensiveness before and at the beginning of the treatment, as on the other cornea of these patients the pigmentation progressed.

The studies revealed repigmentation of the third eyelid margin in 11 patients. It is difficult to estimate the complete repigmentation of the third eyelid margin. Bigelbach (7) observed complete repigmentation of nictitating membrane after long-term treatment. In the presented study, the repigmentation of the third eyelid margin seemed complete in many cases but a detailed examination with the use of computer photos enabled to notice very little depigmentation spots. Therefore, the decreased depigmentation was determined as repigmentation.

In conclusion, the studies proved that 0.02% ophthalmic drops of tacrolimus have been effective topically in canine chronic superficial keratitis treatment. A treatment response was visualised by reduced granulation tissue and corneal neovascularisation. Still in some cases tacrolimus failed to inhibit the pigmentation formation.

References


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