Histologic characteristics and local cellular immunity of the gland of the third eyelid after topical ophthalmic administration of 2% cyclosporine for treatment of dogs with keratoconjunctivitis sicca

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Objective—To evaluate the efficacy of topical administration of a 2% solution of cyclosporine (CsA) for treatment of dogs with keratoconjunctivitis sicca (KCS) and to correlate results with histopathologic characteristics and local cellular immunity of the gland of the third eyelid.

Animals—24 dogs with bilateral KCS.

Procedure—Lacrimal secretion was measured, using Schirmer tear test (STT) strips. Leukocyte and T lymphocyte subsets were determined in blood samples. Histopathologic changes as well as CD4+, CD8+, and alpha-naphthyl-acetate esterase-positive (ANAE+) lymphocytes were evaluated.

Results—Clinical signs resolved at the end of 1 month in conjunction with significantly increased STT values, compared with baseline values. Fifteen and 30 days after discontinuation of CsA treatment, a decrease was observed in STT values in both eyes; however, only values for the right eye were significantly different. There was a significant decrease in the number of lymphocytes and ANAE+ lymphocytes 15 and 30 days after discontinuation of CsA treatment, compared with baseline values. Differences were not observed in number of CD4+ lymphocytes among treatment groups. However, there was a significant decrease in number of CD8+ lymphocytes with reversal of the CD4+:CD8+ in both eyes after CsA treatment for 30 days, compared with the control group. Increased secretory activity and decreased lymphocyte infiltration were characteristic histopathologic findings.

Conclusions and Clinical Relevance—Topical administration of a 2% solution of CsA was effective for the treatment of dogs with KCS. Strict follow-up monitoring is required after the cessation of treatment because of the possibility of recurrence of KCS.

Keratoconjunctivitis sicca (KCS) is a common chronic inflammatory and vision-threatening ophthalmic disease of dogs.1,3 Simultaneous dysfunction of lacrimal glands and the gland of the third eyelid result in KCS.5 Keratoconjunctivitis sicca generally is the result of deficiency in the aqueous layer of precorneal tear film leading to an increase in osmolality with the end result of conjunctival and corneal lesions.6,7 Diagnosis is based on results of a Schirmer tear test (STT) of < 8 or < 10 mm/min in conjunction with associated corneal and conjunctival lesions.9,10 Trauma, infectious diseases, chronic gland adenitis, drug toxicity, immune-mediated diseases, and other systemic diseases are believed to be primary causes of KCS in dogs.1,11-13 In many cases, a specific primary cause is never delineated.1,15

More than 30% of dogs with idiopathic KCS may have immune-mediated disorders of the lacrimal glands and the gland of the third eyelid.9 Keratoconjunctivitis sicca in dogs generally is a result of immune-mediated disease of the lacrimal glands that is not a systemic autoimmune disease.11,12 However, significant differences have not been found in proliferation of blood lymphocytes or numbers of CD4+ and CD8+ lymphocytes between dogs with KCS and clinically normal dogs.12 This suggests that KCS may be associated with immune disorders in the lacrimal gland rather than a multisystemic autoimmune disease.5,14 Immunologic similarities have been observed between dogs and humans with idiopathic KCS. In humans, KCS often is a component of multisystemic autoimmune disease (Sjögren's syndrome).22,23 A disorder resembling Sjögren's syndrome has been reported in dogs.24 An increase in interacinar fibrous tissue and multifocal chronic adenitis characterized by acinar atrophy are common histopathologic changes in the lacrimal glands and the gland of the third eyelid in dogs with KCS.1

Topical applications of lubricants, protectants, anti-inflammatory agents, and mucolytic drugs have been widely used in the treatment of dogs with KCS.13,25,26 Success has been achieved by surgical transposition of the parotid duct to provide lubrication to the eyes and to prevent blindness in some animals, but this liquid is not identical to tears.25-27,30

Topical administration of cyclosporine (CsA) has been used for the treatment of dogs with KCS.5,11,13-15 Cyclosporine suppresses activation of T lymphocytes by reducing production of the lymphokine interleukin-2 and formation of specific interleukin-2 receptors by...
helper T lymphocytes.\textsuperscript{3,14} Cyclosporine is effective in alleviating dry eyes in dogs.\textsuperscript{3,11,13,32} Decreased numbers of lymphocytes were detected in blood samples obtained after treatment of both eyes with 2% CsA, compared with values in control dogs. This suggests that CsA mediates peripheral immune suppression and reduces tissue reaction in the lacrimal glands.\textsuperscript{7} The purpose of the study reported here was to evaluate the efficacy of topical administration of 2% CsA on the clinical signs of KCS and to correlate results with histopathologic characteristics and local cellular immunity of the gland of the third eyelid.

Materials and Methods

Animals—Twenty-four mixed-breed dogs with bilateral KCS were obtained from the University of Selcuk Research Animal Facilities. Dogs were selected on the basis that each had STT values of ≤10 mm/min and associated clinical signs of bilateral KCS (conjunctival hyperemia, mucoid discharge, chemosis, and blepharospasm). Mean interval from time of KCS diagnosis until inclusion in the study ranged from 1 to 16 months (mean, 7.4 months). Dogs ranged from 3 to 14 years of age (mean, 8.9 years). Seventeen dogs were females, and 7 dogs were males. All dogs had results of CBC and serum biochemical analyses that were within reference ranges.

Treatment—Dogs were randomly allotted into 4 groups (control group and 3 treatment groups; n = 6 dogs/group). Dogs in the control group did not receive medication and were used to evaluate the histopathologic characteristics and local cellular immunity of the gland of the third eyelid, compared with results for dogs in the treatment groups. Dogs in all treatment groups received topical administration of a 2% solution of CsA for 30 days. For topical administration, 2% CsA was prepared by diluting the 10% solution of CsA used a using extra virgin olive oil. The mixture was filtered through a 0.45-µm filter and dispensed in a dropper bottle. One drop of 2% CsA was applied to each eye of all dogs in each treatment group every 12 hours for 30 days. Dogs in groups 1, 2, and 3 were evaluated at 30, 45, and 60 days, respectively; thus, dogs in groups 2 and 3 were evaluated 15 and 30 days, respectively, after discontinuation of the drug.

Clinical evaluation—Clinical findings were determined by use of direct and indirect ophthalmoscopy, and STT values were measured in all dogs on alternate days throughout the study. Values for STT were obtained after the administration of 2% CsA in the 3 treatment groups. Baseline values for the STT and numbers of blood lymphocytes before administration of 2% CsA in the treatment groups were used for comparisons.

Collection and processing of blood samples—Venous blood samples were collected into heparin-coated tubes before the first day of treatment and on days 30, 45, and 60. Four smears were prepared from the blood sample of each dog and air dried at room temperature (20 C). Two air-dried smears were stained with May-Grünwald-Giemsa, and the other 2 were fixed by incubation in glutaraldehyde-acetone (pH 6.8) at –10 C to detect alpha-naphthyl-acetate esterase (ANAE) specific to T lymphocytes. Following the incubation, which was performed in accordance with the method of Wulf et al.\textsuperscript{16} Nuclear staining was performed by immersion for 10 minutes in 1% methyl green, which was prepared in acetate buffer (pH 4.2). Lymphocytes that had 1 to 5 reddish-brown reaction products (localized granules) were considered to be T lymphocytes. Number of ANAE-positive (ANAE+) lymphocytes was determined by counting 200 lymphoid cells in each specimen, and values were expressed as percentages. Number and proportion of leukocytes were determined by counting at least 200 leukocytes on May-Grünwald-Giemsa-stained smears; values of various cell types were expressed as percentages.

Collection and processing of tissue samples—Tissue samples of the gland of the third eyelid were obtained from both eyes of each dog in the control group. Dogs were anesthetized for collection of tissue samples. Identical biopsy specimens were collected from dogs in treatment groups 1, 2, and 3 at days 30, 45, and 60, respectively. Comparisons were made between treatment and control groups.

Each biopsy specimen was cut at a thickness of 12 µm, divided into 3 pieces. One of the pieces was wrapped in aluminum foil, immersed in tubes filled with n-hexane, and frozen in liquid nitrogen for use in enzymatic immunohistochemical detection of CD4+ and CD8+ lymphocytes. Frozen specimens were cut at a thickness of 12 µm, using a cryostat. We strictly adhered to the manufacturer’s recommendations for dilution of primary\textsuperscript{a} and secondary\textsuperscript{a} monoclonal antibodies and incubation periods. Peroxidase was detected by use of the method of Kolbjørnsen et al.\textsuperscript{18} Cell nuclei were stained by immersion in 1% methyl green prepared in 0.1M acetate buffer (pH 4.2), and specimens were mounted by use of synthetic medium.\textsuperscript{1} Specimens were observed at high magnification (1,000×), and cells that had morphologic characteristics of lymphocytes and brownish staining of the cellular membrane were considered to have positive results for the primary antibody (CD4+ or CD8+) that was used.

The second piece of each biopsy specimen was fixed in formal-sucrose (pH 6.8) for 21 hours and stored in Holt solution for an additional 21 hours. It then was cut at a thickness of 12 µm. Detection of ANAE+ lymphocytes was performed, by use of the method of Wulf et al.\textsuperscript{16} Cells with morphologic characteristics of lymphocytes and that had a positive reaction for T lymphocytes were classified as ANAE+ lymphocytes. Cell counts were performed in 10 randomly selected areas of each specimen, by use of an ocular square-micrometer (10 × 10 squares). Results were expressed as number of cells per unit area; unit area was 1.44 × 10⁴ µm².

The third piece of each biopsy specimen was fixed in neutral-buffered 10% formalin and embedded in paraffin, using routine histologic techniques. Tissue blocks were cut at a thickness of 7 µm, stained with Crossman trichrome stain, and submitted for histologic examination.\textsuperscript{19}

Statistical analysis—An ANOVA was used to evaluate STT values, values for lymphocytes in blood samples, and values for T-lymphocyte subsets in tissue samples within and among the groups. The Scheffe test and a statistical program were used to compare values among the groups. Values of P ≤ 0.05 were considered significantly different.

Results

Clinical findings and tear production—Clinical signs observed before initiation of treatment resolved after topical administration of 2% CsA; interval until resolution varied among the clinical signs. There was not a significant difference in the efficacy of 2% CsA on the basis of age, sex, and body weight of the dogs. Recurrence of clinical signs was not observed in treatment groups 2 and 3 by 15 and 30 days, respectively, after discontinuation of the drug.

Values for STT before treatment, after administration of CsA for 30 days, and 15 and 30 days after cessation of treatment were determined (Table 1). In
group 1, mean ± SD STT values increased significantly between baseline values and values at the end of 30 days of CsA administration in the right (baseline, 8.50 ± 0.72 mm; 30 days, 13.50 ± 0.43 mm; increase of 58.8%; P < 0.001) and left (baseline, 7.50 ± 1.20 mm; 30 days, 13.83 ± 0.87 mm; increase of 84.4%; P = 0.01) eyes. In group 2, mean STT values increased significantly between baseline and completion of 30 days of administration for the right (baseline, 7.33 ± 0.56 mm; increase of 77.3%) and left (baseline, 8.33 ± 0.71 mm; 30 days, 13.33 ± 1.08 mm; increase of 60%) eyes. On day 45 (ie, 15 days after discontinuation of the drug), there was a significant decrease of STT values for the right eye (10.50 ± 0.34 mm; decrease of –23.8%) and a decrease, but not significant, of STT values for the left eye (11.50 ± 0.72 mm; decrease of –15.9%), compared with the corresponding values for day 30. In group 3, STT values increased significantly between baseline and completion of 30 days of administration for the right (baseline, 8.67 ± 0.42 mm; 30 days, 13.83 ± 0.91 mm; increase of 59.5%) and left (baseline, 8.50 ± 0.62 mm; 13.17 ± 0.65 mm; increase of 54.9%) eyes. On day 60 (ie, 30 days after discontinuation of the drug), there was a significant difference in STT values for the right eye (11.33 ± 0.49 mm) but not the left eye (11.00 ± 0.63 mm) of dogs in group 3, compared with corresponding values for day 30.

Leukocyte analysis—Numbers of leukocytes for treatment groups 1, 2, and 3 were determined (Table 2). In group 1, there was a significant increase in number of neutrophils and a significant decrease in number of lymphocytes at day 30, compared with baseline values. There also were significant decreases in the number of ANAE+ lymphocytes at day 30 for groups 1 (P < 0.001) and 2 (P = 0.01), compared with baseline values for each respective group. For group 3, there was a significant (P < 0.001) increase in the number of neutrophils and a significant (P < 0.001) decrease in number of lymphocytes and ANAE+ lymphocytes at day 30, compared with baseline values.

Analysis of tissue samples—Counts of ANAE+ lymphocytes, CD4+ lymphocytes, and CD8+ lymphocytes in the gland of the third eyelid for the right and left eyes of dogs in the control and treatment groups were determined (Table 3). Number of ANAE+ lymphocytes in the gland of the third eyelid of each eye obtained from groups of dogs (6 dogs/group) with KCS that were treated by topical administration of a 2% solution of CsA for 30 days were reported as millimeters per minute.

Table 1: Mean ± SD results of Schirmer tear tests (STT) for groups of dogs (6 dogs/group) with keratoconjunctivitis sicca (KCS) that were treated by topical administration of a 2% solution of cyclosporine (CsA) for 30 days.

<table>
<thead>
<tr>
<th>Group</th>
<th>Date of test*</th>
<th>Right eye</th>
<th>Left eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Baseline</td>
<td>8.50 ± 0.72</td>
<td>7.50 ± 1.20</td>
</tr>
<tr>
<td></td>
<td>Day 30</td>
<td>13.50 ± 0.43</td>
<td>13.83 ± 0.87</td>
</tr>
<tr>
<td>2</td>
<td>Baseline</td>
<td>7.33 ± 0.56</td>
<td>8.33 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>Day 30</td>
<td>13.00 ± 0.97</td>
<td>13.33 ± 1.08</td>
</tr>
<tr>
<td>3</td>
<td>Baseline</td>
<td>8.67 ± 0.42</td>
<td>9.50 ± 0.62</td>
</tr>
<tr>
<td></td>
<td>Day 30</td>
<td>13.83 ± 0.91</td>
<td>13.17 ± 0.65</td>
</tr>
<tr>
<td></td>
<td>Day 45</td>
<td>11.67 ± 1.09</td>
<td>11.50 ± 1.09</td>
</tr>
<tr>
<td></td>
<td>Day 60</td>
<td>11.33 ± 0.68</td>
<td>11.00 ± 0.63</td>
</tr>
</tbody>
</table>

Values are reported as millimeters per minute.

*Tests were conducted before treatment (baseline), after 30 days of treatment (day 30), 15 days after cessation of treatment (day 45), and 30 days after cessation of treatment (day 60).

**Within a treatment group, values in the same column with different superscript letters differ significantly ("a"P < 0.001; "b"P = 0.01; "c"P < 0.05).

Table 2: Mean ± SD number of leukocytes in blood samples obtained from groups of dogs (6 dogs/group) with KCS that were treated by topical administration of a 2% solution of CsA for 30 days.

<table>
<thead>
<tr>
<th>Group</th>
<th>Date of count*</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Eosinophils</th>
<th>Basophils</th>
<th>ANAE+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Baseline</td>
<td>56.17 ± 4.76</td>
<td>3.17 ± 1.05</td>
<td>3.17 ± 0.98</td>
<td>0</td>
<td>71.67 ± 1.89</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 30</td>
<td>35.50 ± 5.41</td>
<td>4.17 ± 0.79</td>
<td>4.33 ± 0.99</td>
<td>0</td>
<td>56.17 ± 1.54</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Baseline</td>
<td>38.33 ± 3.44</td>
<td>3.17 ± 0.40</td>
<td>5.33 ± 1.36</td>
<td>1.00 ± 0.26</td>
<td>57.50 ± 1.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 30</td>
<td>34.50 ± 3.69</td>
<td>3.50 ± 0.76</td>
<td>4.63 ± 1.05</td>
<td>1.33 ± 0.33</td>
<td>42.83 ± 3.18</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Baseline</td>
<td>37.83 ± 1.91</td>
<td>5.17 ± 0.54</td>
<td>5.50 ± 1.12</td>
<td>0.67 ± 0.21</td>
<td>60.00 ± 2.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 60</td>
<td>22.67 ± 1.61</td>
<td>3.67 ± 0.61</td>
<td>5.67 ± 1.38</td>
<td>0.67 ± 0.21</td>
<td>36.00 ± 2.24</td>
<td></td>
</tr>
</tbody>
</table>

Values are reported as percentages.

*Within a treatment group, values in the same column with different superscript letters differ significantly ("a"P < 0.05; "b"P < 0.001; "c"P = 0.01).

ANAE+ = Alpha-naphthyl-acetate esterase-positive.

*See Table 1 for remainder of key.

Table 3: Mean ± SD lymphocyte counts in the gland of the third eyelid of each eye obtained from groups of dogs (6 dogs/group) with KCS that were not treated (control dogs) or were treated by topical administration of a 2% solution of CsA for 30 days.

<table>
<thead>
<tr>
<th>Group</th>
<th>ANAE+</th>
<th>CD4+</th>
<th>CD8+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right eye</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.17 ± 0.7</td>
<td>2.00 ± 0.4</td>
<td>3.17 ± 0.5</td>
</tr>
<tr>
<td>1</td>
<td>3.17 ± 0.6</td>
<td>2.00 ± 0.5</td>
<td>1.17 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>3.50 ± 0.4</td>
<td>2.20 ± 0.2</td>
<td>1.50 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>3.83 ± 0.7</td>
<td>2.00 ± 0.5</td>
<td>1.83 ± 0.3</td>
</tr>
<tr>
<td>Left eye</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.00 ± 1.3</td>
<td>1.75 ± 0.5</td>
<td>3.25 ± 0.9</td>
</tr>
<tr>
<td>1</td>
<td>3.83 ± 1.4</td>
<td>2.23 ± 0.4</td>
<td>1.50 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>4.17 ± 0.5</td>
<td>2.50 ± 0.2</td>
<td>1.67 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>4.00 ± 1.0</td>
<td>2.67 ± 0.5</td>
<td>1.33 ± 0.2</td>
</tr>
</tbody>
</table>

Values reported represent number of cells/1.44 X 10⁶ µm². Cell counts were obtained on day 30 (ie, after 30 days of CsA treatment) for group 1, on day 45 (ie, 15 days after cessation of CsA treatment) for group 2, and on day 60 (ie, 30 days after cessation of CsA treatment) for group 3.

*Within a column, values with different superscript letters differ significantly ("P < 0.05).
was higher, but not significantly, in the gland of the third eyelid for both eyes of the control group, compared with numbers for the various treatment groups. Although we did not detect a significant difference in the total number of CD4+ lymphocytes between the control and treatment groups, there was a significant decrease in the number of CD8+ lymphocytes in the treatment groups, compared with values for the control group. Number of ANAE+, CD4+, and CD8+ lymphocytes did not differ significantly among treatment groups. Numbers of CD4+ and CD8+ lymphocytes did not differ significantly on the basis of age, sex, and body weight of the dogs in treatment and control groups.

Severe glandular degeneration and atrophy were observed in the gland of the third eyelid in both eyes of all dogs in the control group. Fibrosis and an increase in adipose tissue were evident in the connective tissue trabeculae. Tubular dilatation was observed in lacrimal ducts (Fig 1). Two dogs had noticeable mononuclear cell infiltration, and 1 dog had mild perilobal lymphoid cell infiltration.

Degeneration was more severe in the superficially localized secretory units in these dogs.

In treatment group 1, 4 dogs had normal histologic characteristics of the gland of the third eyelid in both eyes (Fig 2). Partial glandular degeneration, tubular dilatation, fibrosis, an increase in adipose tissue, and lymphocytic infiltration were observed in the gland of the third eyelid in the left eye of 2 dogs.

In treatment group 2, there were increased amounts of adipose and connective tissues with decreased numbers of secretory units (Fig 3). Vascular hyperemia, increase in vascularization, dilated tubuli, lymphocytic and macrophage infiltration, and glandular atrophy were predominant findings. Secretory units with high secretory activity were observed in the gland of the third eyelid in the left eye of 2 dogs. Ductular epithelium was flattened as a result of retention of secretions. Vascular hyperemia, epithelial desquamation, and tubular dilatation also were evident in the glandular tissue.

In treatment group 3, 4 dogs had varying degrees of glandular degeneration and atrophy in both eyes.
Two dogs had actively secreting glands with partial glandular degeneration and atrophy. Epithelial desquamation and flattened ductular epithelium also were evident in these glands (Fig 4). Results for dogs in group 3 were similar to those of dogs in group 2.

Adverse reactions—Topical administration of 2% CsA did not result in adverse reactions during the treatment and follow-up periods. During the first 10 days of treatment, dogs did have a tendency to rub their face and eyes after each application; however, this behavior disappeared when the clinical signs associated with corneal and conjunctival findings before treatment resolved at various intervals. There was a significant difference in the efficacy of 2% CsA after topical administration of 2% CsA. None of the dogs had similar histologic characteristics to those of normal glands. The STT values were significantly increased 30, 45, and 60 days after initiation of treatment in both eyes of dogs with KCS in treatment groups 1, 2, and 3, compared with baseline values for each respective group.

Recurrence of KCS may be seen after discontinuation of the drug. Although there was a gradual decrease in STT values, recurrence of clinical signs was not observed 15 or 30 days after discontinuation of CsA in treatment groups 2 and 3, respectively; however, histopathologic appearance of the gland of the third eyelid was more similar to the normal gland in the eyes of dogs in group 1 than those of groups 2 and 3. This may in turn result in recurrence of clinical signs long (> 30 days) after discontinuation of the drug. Therefore, it is necessary that dogs with KCS that are treated with CsA be strictly monitored.

Degeneration of tubuloalveolar secretory units and tubular epithelial cells of the main lacrimal gland and gland of the third eyelid has been reported in dogs with KCS. In mice with xerostomia and xerophthalmia, CD4+ and CD8+ lymphocytes have been observed in the stroma of salivary and lacrimal glands. Lymphocytic infiltration and an increase in the number of plasma cells also are common histologic findings in patients with Sjögren’s syndrome, and it has been suggested that damage and dysfunction of the glandular epithelium is activated mainly by CD4+ T lymphocytes through the induction of apoptosis. Degenerative changes can result from infiltration of CD4+ and CD8+ lymphocytes around the secretory units and duct system of lacrimal glands. Many clinical signs of dry eyes result from systemic autoimmune disease affecting lacrimal glands as well as local disorders attributable to deficiency in androgen hormones, especially in menopausal females. Because of the possible role of disorders in local immunity of the gland, it may be of paramount importance to use immunosuppressive agents in the treatment of dogs with KCS. It is not clear whether CsA increases secretion of the lacrimal glands by suppressing inflammatory reactions or stimulates tear production through direct or indirect mechanisms.

The drug quickly penetrates into lacrimal gland tissues after topical application, and CsA mediates suppression of peripheral immune responses and subsequently reduces tissue reactions in the lacrimal glands.

Controversy exists among investigators regarding whether subsets of T or B lymphocytes mediate initiation of degeneration of lacrimal gland cells. Infiltration of CD4+ lymphocytes in lacrimal glands also is observed in patients with Sjögren’s syndrome. Lesions in the lacrimal glands of several strains of mice autoimmune for Sjögren’s syndrome revealed differing dominant T-lymphocyte subsets depending on the strain of mice. Therefore, regression of clinical signs of systemic autoimmune disease is improved by the use of combined treatment with anti-CD4 and anti-CD8 monoclonal antibodies. However, in the study reported here, the number of CD8+ T lymphocytes in unit areas was more than that of CD4+ lymphocytes in the gland of the third eyelid in both eyes in dogs of the control group. Decrease in the number of CD8+ lymphocytes, compared with the number of CD4+ lymphocytes, in the treatment groups and an increase in
the number of CD8+ lymphocytes, compared with the number of CD4+ lymphocytes, in the control group in our study confirm that CD8+ lymphocytes are more effective than CD4+ lymphocytes in epithelial destruction. This is in agreement with the reported cytotoxic effect of CD8+ lymphocytes located around acinar epithelial cells adherent to the alpha E beta 7 molecule, leading to apoptosis with the resultant effect of secretory dysfunction of exocrine glands.\(^5\)\(^6\)\(^7\) There is lack of information on cell populations, cell types, and cell ratios in lacrimal glands of clinically normal dogs. In lacrimal glands of humans, 59.9% of mononuclear cells are plasma cells, which synthesize IgA, and 40.3% are T lymphocytes, with the proportion of CD8+ to CD4+ lymphocytes ranging from 14.7 to 25.27%.\(^8\)\(^9\) In the study reported here, mean number of CD8+ and CD4+ lymphocytes per unit area in the gland of the third eyelid of the right eyes of dogs with KCS was 3.17 ± 0.5 and 2.0 ± 0.4, respectively, whereas it was 3.25 ± 0.9 and 1.75 ± 0.5, respectively, for the gland of the third eyelid of the left eyes. In our study, a significant decrease was observed for all treatment groups in CD8+ lymphocyte counts with reversal of the CD4+:CD8+ in the gland of the third eyelid of both eyes at the end of the 30-day treatment period, compared with values for the control group at the same time. Number of CD4+ lymphocytes in the gland of the third eyelid of both eyes of the dogs with KCS in the treatment groups was identical to that of dogs in the control group. The decrease in CD8+ lymphocyte counts at day 30 for dogs in group 1 was similar to that of the dogs in groups 2 and 3 at 15 and 30 days after discontinuation of CsA treatment, respectively. Decreases that were not significant were observed in ANAE+ lymphocyte counts in the gland of the third eyelid for both eyes of the treatment groups, compared with values for the control group. It is noteworthy that this nonsignificant decrease was observed in conjunction with a significant decrease in the ANAE+ lymphocyte counts in blood.

The CD4+/CD8+ (ie, ratio of helper-inducer T cells and cytotoxic-suppressor T cells) in lymphocytes in blood samples is approximately 2.1 in humans\(^10\) and between 2.4:1 and 2.8:1 in dogs.\(^11\) This ratio increases in humans with systemic autoimmune diseases.\(^12\) Nevertheless, it has been reported\(^13\) that there is no difference between the total numbers of CD4+ and CD8+ lymphocytes in blood samples of dogs with KCS. This may explain the reason that the disease develops as a result of diminishing local immune reactions in the lacrimal glands rather than disorders of systemic immunity. It is important to take this fact into account when evaluating results, because lymphocyte subsets vary among individuals.\(^14\)\(^15\) It may require from 1 to 3 months for administration of 2% CsA to decrease peripheral lymphocyte proliferation and suppression of peripheral cellular immunity in dogs with KCS.\(^16\) In the study reported here, there was a significant decrease in the number of lymphocytes in the blood, compared with baseline values, after 30 days of treatment for dogs in group 1. On the other hand, the decrease in number of blood lymphocytes was not significant in group 2. However, the decrease in number of blood lymphocytes was significant in group 3. Number of ANAE+ lymphocytes in blood samples revealed a significant gradual decrease within and among the treatment groups.

It is clear that treatment with CsA caused substantial changes in the number of lymphocytes in blood samples. Although there was a significant increase in neutrophil counts, lymphocyte counts decreased significantly in CsA-treated dogs of group 1. There also were significant decreases after treatment in the number of ANAE+ lymphocytes in blood samples for dogs in groups 1 and 2, compared with baseline values for each respective group. However, by day 60 of the study, neutrophil counts increased significantly, whereas blood lymphocyte and ANAE+ lymphocytes counts decreased significantly, compared with baseline values, for dogs in group 3. The significant decrease in the lymphocyte population of groups 1 and 2 was accompanied by a significant decrease in number of ANAE+ lymphocytes in blood samples of groups 1, 2, and 3. On the basis of these results, it is reasonable to state that adverse effects would result from the systemic immunosuppressive effect of the drug during long-term use.

It was concluded that topical administration of 2% CsA was effective in the treatment of dogs with KCS. Long-term use of the drug may be required with strict follow-up monitoring after the cessation of treatment, because the possibility for recurrence of KCS exists. However, adverse effects that result from the systemic immunosuppressive effect of CsA during long-term use should be considered when planning a treatment protocol.

References


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Footnotes:

\(^a\)Sandimmune, Sandoz Pharma Ltd, Basel, Switzerland.
\(^b\)Syrlil-MF, Costar Corp, Cambridge, Mass.
\(^c\)HR Cryostat, SLEE, London, UK.
\(^d\)Rat anti canine CD4 (MCA 10386), Serotec Co, Oxford, UK.
\(^e\)Rat anti canine CD8 (MCA 10396), Serotec Co, Oxford, UK.
\(^f\)Mouse anti-rat, IgG2A: HRP (MCA 278P) for CD4, Serotec Co, Oxford, UK.
\(^g\)Mouse anti-rat Ig G1: HRP (MCA 194P) for CD8, Serotec Co, Oxford, UK.
\(^h\)Entellan, Merck and Co, Darmstadt, Germany.
\(^i\)SPPS 6.0, SPSS Inc, Chicago, Ill.


