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# Effect of Topical Cyclosporin A on Conjunctival T Cells in Patients with Secondary Sjögren's Syndrome

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The effect of topical cyclosporin A on conjunctival T cells was studied in nine patients with secondary Sjögren's disease. Patients had conjunctival biopsies performed before and after a 6-week course of topical cyclosporin. Epithelium and substantia propria in the Sjögren's patients before treatment showed significantly more CD4+ cells than specimens taken from nine age- and sex-matched controls. Following treatment with topical cyclosporin, there was a significant reduction in the number of CD4+ cells in both the conjunctival epithelium and substantia propria. Despite the fact that the treatment resulted in immunopathological improvement, the clinical benefit was not as favorable. Our results suggest that topical cyclosporin may have a local immunosuppressive effect on the conjunctiva in patients with Sjögren's disease.

**Key Words:** Cyclosporin—Sjögren's syndrome—Monoclonal antibody—Conjunctival biopsy—T-cells.

Cyclosporin A is a cyclic polypeptide of 11 amino acids. It is insoluble in water but can be dissolved in olive oil or sesame oil at 60°C. It inhibits T lymphocyte response to mitogens or antigens and lymphokine production by activated T lymphocytes (1,2). It has also been shown to block the production of interleukin 2 by activated T lymphocytes (3). In contrast, it has no effect on T suppressor lymphocytes (2). The usefulness of cyclosporin A in tissue transplantation has been extensively investigated. It has been shown to be effective in increasing the survival time of renal (4,5), liver (6), and cardiac (7) transplants. It has also been used with success in preventing graft versus host disease in patients after bone marrow transplantation (8,9).

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In patients with chronic intraocular inflammation, systemic cyclosporin has been used with some success, although side effects, particularly nephrotoxicity, were seen in a significant proportion of patients (10). Systemic cyclosporin has also been used with varying success in the prevention of corneal allograft rejection in high-risk patients (11,12), in the treatment of patients with Behçet's disease (13–15), and in the treatment of Mooren's ulcer (16). Recurrence of disease is not infrequent after discontinuing treatment, and long-term treatment may be necessary (10). In an effort to overcome the side effects associated with long-term systemic cyclosporin, workers have recently been studying the effects of topical cyclosporin A on immunoreactive cells at a local level (17).

Sjögren's syndrome is an autoimmune disease characterized by lymphocytic infiltration of the exocrine glands and polyclonal B lymphocyte proliferation (18). It is generally accepted that the principal cell of this inflammatory infiltrate is an activated T lymphocyte bearing the T helper-inducer phenotype (19). This finding, together with the fact that cyclosporin appears to have a inhibitory effect on these cells, prompted us to study the effect of topical cyclosporin on a group of patients with secondary Sjögren's syndrome. We report our results on the use of topical cyclosporin in these patients from a clinical and immunocellular perspective.

## PATIENTS AND METHODS

### Patients

Patients were eligible for admission to the study if they had a severe tear film defect in association with a diagnosed connective tissue disorder. The

diagnosis of connective tissue disease was made by either a clinical immunologist or rheumatologist on the basis of clinical, laboratory, and radiological findings (Table 1). For the purpose of the study, patients were deemed to have a severe tear film defect when they had a Schirmer test done with topical anaesthesia of 0 on two separate occasions, abnormal tear film break up time (<5 s), had positive rose bengal staining of conjunctiva and cornea, and had symptoms not controlled on maximum ocular lubricants. Six of the patients had punctal occlusion performed on both upper and lower lids in the past. All patients were using preservative-free ocular lubricants. Six of the patients also had xerostomia, defined as symptoms of dry mouth with associated dysphagia.

The following were excluded from the study: uniocular patients, those who had received either topical or systemic cyclosporin in the past, and anyone who was currently on immunosuppressive therapy. Informed consent was obtained from each patient.

On entry into the study, patients had a full-thickness inferonasal bulbar conjunctival biopsy performed 4 mm from the limbus under local anaesthesia. Two weeks later, when the eye had settled, patients were started on 2% cyclosporin A drops in olive oil to be used four times daily in both eyes. During the next 6 weeks, patients were seen on three occasions. At each visit, patients were questioned as to whether their symptoms had improved, deteriorated or had remained static. Symptoms of dryness, grittiness, and burning were scored on a four-point scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe). Pretreatment scores were compared to the final examination scores. An attempt was made to assess the effect of treatment on the degree of epithelial damage as indicated by staining. The amount of rose bengal staining was assessed as absent, mild, moderate, or severe and scored 0, 1, 2, or 3, respectively, as described by van Bijsterveld (20). Any evidence of toxicity that could have been attributed to the treatment was noted. In particular, evidence of corneal epithelial toxicity and allergic reactions was looked for. No attempt was made to score these adverse events. At the end of the 6 weeks of treatment, a conjunctival biopsy adjacent to the previous site was repeated and the treatment discontinued. Nine age- and sex-matched patients undergoing cataract or strabismus surgery also had conjunctival biopsies taken in an identical manner for comparison. These patients all had normal Schirmer testing, had no evidence of rose bengal staining, and, apart from either cataract or strabismus, had a normal ocular examination. None of the control patients were on any ocular medications.

**TABLE 1.** *Clinical findings*

| Patient | Age | Sex | Xerophthalmia | Xerostomia | Diagnosis |
|---|---|---|---|---|---|
| 1 | 59 | F | + | + | SLE |
| 2 | 30 | F | + | + | MCTD |
| 3 | 81 | F | + | + | RA |
| 4 | 57 | F | + | + | RA |
| 5 | 53 | F | + | − | RA |
| 6 | 64 | F | + | + | MCTD |
| 7 | 73 | M | + | − | RA |
| 8 | 72 | M | + | + | RA |
| 9 | 39 | F | + | − | RA |

SLE, systemic lupus erythematosus; MCTD, mixed connective tissue disease; RA, rheumatoid arthritis.

### Methods

Biopsy material was snap frozen using L.I.P. Freeze (Equipment and Services, Galway, Ireland) instant freezing aerosol. Seven-micron frozen sections were air dried for 4 h. Subsequently, sections were fixed in acetone for 10 min at room temperature and allowed to air dry for 5 min, following which they were immersed in tris buffered saline (TBS). The primary antisera (Ortho Pharmaceuticals, Dublin, Ireland) were applied for 1 h (anti-CD4 at a dilution of 1 in 50 and anti-CD8 at a dilution of 1 in 200). Sections were then washed three times in TBS for 15 min each wash and then incubated with rabbit anti–mouse antibody (Dako, Copenhagen, Denmark) 1 in 200 for 30 min. Following subsequent washing for 15 min, sections were incubated with avidin biotin complex for 30 min and following further washing were developed in diamino benzidine. The primary antibodies used were the CD4 antibody to identify T-helper/inducer cells (CD4+ cells) and the CD8 antibody to identify T-suppressor/cytotoxic cells (CD8+ cells). Positive controls were performed with fresh tissue obtained from tonsillectomy specimens and were processed in the same way as described for the conjunctival specimens. Negative controls consisted of replacing the primary antibody with TBS. Sections were counter stained with haematoxylin, and were then dehydrated, cleared, and mounted in DPX (a xylene-based neutral mounting medium). The processed sections were read in a masked fashion using a Nikon microscope with an attached eye piece graticule. Using a ×40 objective, the number of cells labelled with each monoclonal antibody was counted for at least 10 different fields in both the epithelium and substantia propria. Cells were considered positive if they showed a dense dark blackbluish ring on the cellular membrane. The total number of stained cells was expressed as the number of cells per $mm^2$.

Means and standard errors of the mean were calculated for both cell types in the normals and in the Sjögren's' group before and after treatment. Comparisons between groups were analyzed using the Wilcoxon two-sample rank sum test.

## RESULTS

Nine patients with Sjögren's disease were entered into the study. The mean cell numbers in the epithelium and substantia propria are shown in Tables 2 and 3. There was a significantly greater number of CD4+ cells in the epithelium and substantia propria in the Sjögren's group before treatment when compared with the normals. There was no significant difference in the number of CD8+ cells in the epithelium of the normals versus the pre treatment Sjögren's and although there was a greater number of CD8+ cells in the substantia propria, this was not statistically significant.

Following treatment, there was a significant reduction in CD4+ cells, in both the epithelium and substantia propria in the Sjögren's patients. There was no significant change in the number of CD8+ cells following treatment.

Table 4 shows the effect of treatment on the chosen clinical parameters. There was a significant improvement in the symptom of dryness, but there was also a definite deterioration in the symptom of burning. This was experienced by all patients. When questioned about this, all patients felt that this was directly related to the drops. All patients experienced burning following instillation of the drops lasting from a few minutes to a maximum of 30 min. Patients were asked if this burning would discourage them from continuing treatment after the study period. Seven of the nine said they would prefer not to continue with the treatment on completion of the study because of the discomfort they experienced after instilling the drops. Two patients developed a "hurricane" epitheliopathy that persisted throughout the course of treatment. In both patients, the epitheliopathy resolved within 2 weeks of discontinuing the treatment. There were no other side effects noted.

## DISCUSSION

The aim of this study was to look at conjunctival lymphocyte subpopulations in patients with Sjögren's disease before and after treatment with topical cyclosporin. We have shown that a 6-week course of topical cyclosporin significantly decreased the numbers of CD4+ cells in both the epithelium and substantia propria. Dalavanga et al. (21) demonstrated a reduction in the number of CD4+ cells in labial minor salivary gland tissue following systemic cyclosporin A treatment in a group of patients with Sjögren's syndrome. However, like the authors of the present study, they were disappointed in the clinical improvement produced with the treatment. In a study on the effects of topical cyclosporin in nickel hypersensitivity, Aldridge et al. (22) demonstrated a marked diminution in lymphocyte infiltration in skin biopsies adjacent to contact reactions following treatment with topical cyclosporin 5%. They were unable to detect systemic absorption of the drug and therefore suggested that the effect was produced by local alteration of the immune response.

There have been few reports on the use of topical cyclosporin in the treatment of Sjögren's syndrome (23). In a series of 36 sequential cases of canine keratoconjunctivitis, Kaswan et al. (23) demonstrated a significant increase in tear production and a progressive reduction of superficial corneal neovascularisation with topical cyclosporin usage. Additional benefits that were seen with the treatment included reduced mucopurulent conjunctivitis, rapid healing of persistent corneal ulcers, and reduced dependence on frequent topical lubricants. There was no attempt made to look at the effect of the treatment at a cellular level.

Our results provide further evidence that topical cyclosporin may have a local immunoregulatory effect on human conjunctiva. Antigen stimulus to T-cells is required continually to maintain an active immune response (24). In interfering with the early helper T-cell response, topical administration of cyclosporin can interrupt an ongoing immune reaction. We believe that topical cyclosporin may work

**TABLE 2.** *Lymphocyte subpopulations in conjunctival epithelium*[a]

| Cell type | Normals (n = 9) | Sjögren's (n = 9) | | p (before vs after treatment) |
|---|---|---|---|---|
| | | Before treatment | After treatment | |
| T helper/inducer cells (CD4+) | 2.42 ± 0.31 (1.6–3.1) | 5.61 ± 1.63 (3.4–7.1) | 2.78 ± 0.29 (1.5–3.5) | 0.018 |
| T cytotoxic/suppressor cells (CD8+) | 3.13 ± 0.78 (1.8–4.8) | 2.87 ± 0.96 (1.3–5.1) | 3.27 ± 0.81 (2.1–5.2) | 0.17 |

[a] Number of cells/mm$^2$ ± SEM. The range is given in brackets.

**TABLE 3.** *Lymphocyte subpopulations in conjunctival substantia propria*[a]

| Cell type | Normals (n = 9) | Sjögren's (n = 9) | | p (before vs after treatment) |
|---|---|---|---|---|
| | | Before treatment | After treatment | |
| T helper/inducer cells (CD4+) | 6.92 ± 1.71 (3.9–8.7) | 21.68 ± 3.12 (15.2–24.7) | 10.49 ± 1.49 (8.7–13.1) | 0.02 |
| T cytotoxic/suppressor cells (CD8+) | 8.34 ± 2.12 (6.1–10.9) | 13.15 ± 2.34 (9.1–16.4) | 11.87 ± 1.57 (8.8–13.3) | 0.29 |

[a] Number of cells/mm² ± SEM. The range is given in brackets.
NS, not significant.

in patients with Sjögren's syndrome because of its local immunosuppressive effect and not because of systemic absorption. Studies have shown that the topical application of drops does not produce detectable levels in the plasma (25,26). Furthermore, Foets et al. (27) demonstrated that topical cyclosporin applied to keratoplasties in rabbits protected the graft when applied to the recipient eye but not when applied to the fellow eye.

The commonest side effect noted in the study was burning. All patients complained of this, although no patient discontinued treatment because of it. However, our study population may not be a representative group, as all our patients were highly motivated in their efforts to find a treatment which would help alleviate their symptoms. Two patients developed an epitheliopathy that resolved on discontinuation of the treatment when they had completed the study. It is not certain whether this epitheliopathy is due to a direct toxic effect of the cyclosporin or the olive oil on the corneal epithelium or whether it is a feature of deficient corneal wetting. Versura (28) used scanning electron microscopy to look at the effect of cyclosporin on the corneal epithelium of rabbits. She detected small focal areas where the normal arrangement of epithelial cells was lost. These changes were present in both the cyclosporin treated rabbits and in those receiving olive oil only, suggesting that the oil as a vehicle may be responsible for the epithelial changes seen. It is also noteworthy that Kaswan (23) noted a decrease in the frequency of irritative reactions when corn oil was used instead of olive oil. The effects of long-term usage of topical cyclosporin has not been studied, but it has been shown that topically applied cyclosporin does not inhibit corneal healing in the rabbit and guinea pig eye with short-term usage (29).

We have demonstrated that the topical administration of 2% cyclosporin four times daily produces an immunopathological improvement in patients with Sjögren's disease. However, the clinical benefit was disappointing, mainly because of local irritation following use of the drug. This may have been related to the vehicle used to administer the drug. The use of cyclosporin in this condition clearly warrants further evaluation, and the results of a multicenter clinical trial evaluating the use of 2% cyclosporin ointment in patients with keratoconjunctivitis sicca, currently underway in the United States, are keenly awaited.

**TABLE 4.** *Change in clinical parameters following treatment*[a]

| | Score change | No. of patients |
|---|---|---|
| Dryness | 0 | 2 |
| | +1 | 7 |
| | p = 0.016 | |
| Grittiness | −1 | 1 |
| | 0 | 2 |
| | +1 | 6 |
| | p = 0.125 | |
| Burning | −1 | 3 |
| | −2 | 4 |
| | −3 | 2 |
| | p = 0.004 | |
| R.B. Staining | +1 | 3 |
| | 0 | 4 |
| | −1 | 2 |
| | p = 1.00 | |

[a] For each parameter, the difference in scores before and after treatment is given (+, improvement; −, deterioration). A two-tailed sign test was used to determine significance.

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