Original Article

Topical Cyclosporine A for Mustard Gas Induced Ocular Surface Disorders

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Abstract

Purpose: To evaluate the effectiveness of topical cyclosporine A 0.05% for treatment of mustard gas-induced ocular surface disorders with special attention to conjunctival goblet cell density in patients with severe dry eye.

Methods: This prospective clinical study included 20 eyes of 20 patients previously exposed to mustard gas with dry eye syndrome unresponsive to artificial tears. Before and after treatment with topical cyclosporine A 0.05% twice daily for 3 months, subjects were evaluated for improvement in symptoms using the ocular surface disease index (OSDI) and signs by tear breakup time (TBUT), Schirmer test and measurement of superior bulbar conjunctival goblet cell density. Limbal stem cell deficiency (LSCD) and the degree of corneal squamous cell metaplasia were also assessed before and after treatment.

Results: Before treatment, mean OSDI score, Schirmer test I value and mean TBUT were 42.8 ± 6.1, 4.2 ± 1.2 mm and 2.5 ± 1.3 s, respectively. After 3 months of treatment with topical cyclosporine A, these scores reached 36.4 ± 5.2, 5.8 ± 1.6 mm and 4.9 ± 2.1 s, respectively showing a statistically significant improvement ($P < 0.001$) in all parameters. Mean goblet cell density was 23.3 ± 17.1/high power field (hpf) at baseline which was significantly increased to 47.7 ± 16.1/hpf at the end of the study ($P < 0.001$). There was no improvement, however, in corneal conjunctivalization, LSCD and the degree of corneal squamous cell metaplasia based on impression cytology reports ($P > 0.05$).

Conclusion: Treatment with topical cyclosporine A 0.05% in patients with severe dry eye due to mustard gas injury increases goblet cell density in the bulbar conjunctiva and improves symptoms of the disease.

Keywords: Cyclosporine A; Dry Eye Disease; Goblet Cell Density; Impression Cytology; Mustard Gas

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INTRODUCTION

Exposure to the highly toxic mustard gas causes late complications such as dry eye and decreased tear meniscus.[1-4] Chronic and delayed-onset keratopathy induced by sulfur mustard are characterized by involvement of the conjunctiva, limbus and cornea. Chronic blepharitis, meibomian gland dysfunction, limbal ischemia, limbal stem cell deficiency (LSCD) as well as corneal neovascularization and scarring are different features of mustard gas keratopathy.[4,5] A decrease in conjunctival goblet cells plays a pivotal role in tear film instability, as mucin produced by goblet cells increases tear viscosity and helps the tear film not to break up.[6-9] In recent years, treatment strategies targeting inflammation of the corneal surface as the pathophysiologic basis of dry eye disease have greatly been emphasized.[9-11]

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Cyclosporine A has been shown to be effective in reducing dry eye symptoms and there are studies supporting the role of this medication in increasing goblet cell density and immune modulation of the bulbar conjunctiva in patients with different grades of the disease.[12,13]

Herein, we assessed the effect of topical cyclosporine A (CsA) 0.05% on ocular surface disorders as well as goblet cell density in the bulbar conjunctiva of patients suffering from severe resistant dry eye disease due to prior exposure to mustard gas. The effect of the medication on corneal squamous metaplasia and LSCD was also evaluated. It was hypothesized that the number of conjunctival goblet cells is low in these patients and that topical cyclosporine A could be effective in increasing the density of these mucin producing cells and thus may improve symptoms of the disease.

METHODS

This prospective clinical study consisted of 20 eyes of 20 patients exposed to mustard gas who suffered from severe dry eye unresponsive to conventional treatment strategies such as artificial tears and punctual plugging. Written informed consent was obtained from all participants and all subjects underwent a thorough ocular and slit lamp examination. A detailed history was taken regarding the duration of exposure to mustard gas, dry eye symptoms, systemic and ocular medications, any ocular surgery as well as systemic conditions. All subjects were evaluated using the Ocular Surface Disease Index (OSDI), which consists of 12 questions measuring the severity of dry eye symptoms and the score was calculated using the methods described by the authors.[14] Schirmer test with anesthesia was obtained using a standard Schirmer test strip which was placed in the temporal third of the lower eyelid for 5 min. The tear break up time (TBUT) was also measured after applying a fluorescein strip moistened with a drop of saline. Severe dry eye was defined as having annoying, chronic and/or constant visual symptoms limiting patient activities and Schirmer score of ≤5 mm and TBUT ≤5 s.[9] For each patient only the worse eye was enrolled in the study and all tests were repeated after 3 months of treatment with topical cyclosporine A 0.05% (Restasis®; Allergan Inc., Irvine, CA, USA), twice daily plus preservative-free artificial tears (Artelac Advanced; Bausch and Lomb GmbH, Germany) 6 times daily. Exclusion criteria were active ocular infection, use of any systemic medication interfering with tear production, history of ocular surgery within the past 3 months and hypersensitivity to cyclosporine.

Impression Cytology

Impression cytology samples were taken from the superior bulbar conjunctiva at baseline and 3 months after treatment with topical cyclosporine A. After anesthetizing the eye with 0.5% topical tetracaine hydrochloride, cellulose acetate filters (Whatman GmbH, Hahnestraβe 3-D-37586, Dassel, Germany) with pore size of 0.2 μm were cut into trapezoidal pieces and applied to the bulbar surface straddling the limbus with one half of the paper covering the cornea and the other half covering the conjunctiva. After gentle pressure on the papers for a few seconds, they were removed and sent into a fixative liquid composed of glacial acetic acid, 40% formaldehyde and 70% ethanol. Papers containing cells were then stained with periodic acid-Schiff (PAS) Papanicolaou stain and examined under a light microscope (Olympus BX 41, Tokyo, Japan). Goblet cells were counted in five microscopic fields (objective lens: ×40/0.65) and the mean goblet cell count was calculated for the quadrant per high power field (hpf). Corneal conjunctivalization and LSCD were defined as the presence of goblet cells on the corneal side of the samples. The degree of corneal squamous metaplasia was graded based on scores obtained by cytopathologic changes and nucleus condensation, the degree of inflammatory cell infiltration, cytoplasm color and the degree of keratinization [Table 1]. Corneal squamous cell metaplasia was considered mild, moderate and severe for total points of 3-5, 6-9 and more than 10, respectively.

Data Analysis

Statistical analysis was performed using SPSS software (version 18, SPSS, Inc., Chicago, IL, USA). The nonparametric test (Wilcoxon matched-pairs test) was used for analyzing the data which were not normally distributed and $P < 0.05$ were considered as statistically significant.

Table 1. Cytopathologic criteria for grading corneal squamous cell metaplasia

Points	N/C ratio	Nucleus condensation	Inflammatory cell infiltrate	Cytoplasm color and keratinization
0	1:2	None	None	Blue-green color
1	1:4	Few snakes and pyknosis	Mild	Pinkish (metachromatic), some cells contain keratin filaments
2	1:8	Moderate snakes and pyknosis	Moderate	More cells contain densely packed keratin filaments
3	1:12	Extensive snakes and pyknosis	Severe	Shrunken densely packed keratin filaments

N/C, nucleus/cytoplasm ratio

RESULTS

Nineteen patients out of 20 (95%) initially enrolled subjects with severe dry eye induced by mustard gas completed the study and were included in the statistical analysis. All patients were male subjects with mean age of 49.2 ± 5.3 (range, 42-69) years. Mean duration of exposure to mustard gas was 26.4 ± 2.1 years. Before the study all patients were using artificial tears and 14 of them (73.7%) had undergone punctal plugging. The most common complaints of patients were itching in 15 (78.9%), burning sensation in 14 (73.7%) and foreign body sensation in 12 (63.2%) subjects. Slit lamp examination of the conjunctiva and cornea revealed abnormal findings such as limbal ischemia affecting at least one third of the limbus in 11 (57.9%), different levels of corneal opacity in 13 (68.4%) and corneal vascularization in 10 (52.6%) cases.

No serious adverse effect related to topical cyclosporine A use was reported. Only 2 patients complained from burning sensation which resolved after a few days of therapy. Mean OSDI score before treatment was 42.8 ± 6.1. After 3 months of treatment with topical cyclosporine, it was reduced to 36.4 ± 5.2 ($P < 0.001$). The mean Schirmer test value was also significantly improved from 4.2 ± 1.2 mm at baseline to 5.8 ± 1.6 mm after treatment $P < 0.001$). Mean TBUT at baseline was 2.5 ± 1.3 s which significantly increased to 4.9 ± 2.1 s ($P < 0.001$). Impression cytology revealed insufficient specimen in 2 cases. The presence of goblet cells on the corneal side of the specimen, which was considered as conjunctivalization of the cornea and LSCD, was observed in 15 cases. Corneal squamous cell metaplasia was seen in 14 eyes including mild, moderate and severe metaplasia in 7 (50%) 6 (42.9%) and 1 (8.1%) eyes, respectively. After treatment, there was no improvement in corneal conjunctivalization, LSCD and the degree of corneal squamous cell metaplasia based on impression cytology reports ($P > 0.05$). Mean goblet cell density of all cases was 23.3 ± 17.1/hpf at baseline which increased by 2-fold to 47.7 ± 16.1/hpf at the end of the study [$P < 0.001$, Figure 1]. Most patients (78.9%) showed an increase in the number of goblet cells in their bulbar conjunctiva [Figure 2]. Table 2 summarizes the results of evaluations before and after treatment. There was no statically significant correlation between improvement in OSDI scores and objective scores using Spearman correlation coefficients ($P > 0.05$).

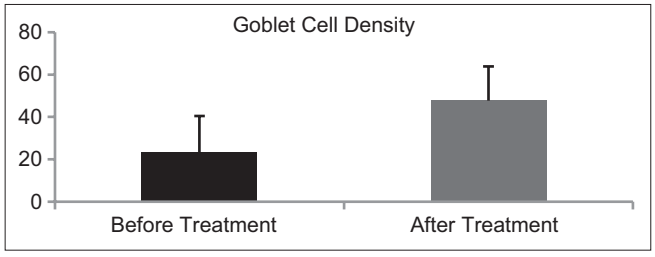

Figure 1. Mean goblet cell density of the patients before and after treatment.

DISCUSSION

Dry eye is a late ocular complication of exposure to mustard gas, the symptoms of which are often severe and persistent influencing many aspects of intoxicated patients' lives.[1-4] In the present study, histopathologic and clinical tests were used to analyze the response to therapy with topical CsA 0.05% in patients with severe dry eye caused by prior exposure to mustard gas. It was found that treatment with CsA 0.05% for 3 months increased goblet cell density in the bulbar conjunctiva in our patients. Moreover, significant improvements in symptoms and clinical tests (OSDI, Schirmer test and TBUT) were observed. Consistent with the results of a study by Baradaran-Rafii et al,[15] LSCD and corneal squamous cell metaplasia were observed based on impression cytology samples from most cases. However, there was no improvement in LSCD and the degree of corneal squamous cell metaplasia at the end of the study proposing that topical CsA 0.05% was not effective in reverting these features of mustard gas keratopathy at least for the treatment period of 3 months.

Dysfunction of ocular surface and lacrimal gland leads to tear film instability in the ocular surface. Although the exact pathophysiologic cause of dry eye syndrome is not known yet, most studies in this regard

Table 2. Dry eye evaluation scores before and after treatment with cyclosporine A

	Before treatment	After treatment
OSDI	42.8±6.1	36.4±5.2
Schirmer I (mm)	4.2±1.2	5.8±1.6
TBUT (s)	2.5±1.3	4.9±2.1
Goblet cell density (/hpf)	23.3±17.1	47.7±16.1

Results are expressed as means±SD. OSDI, ocular surface disease index; TBUT, tear break up time; SD, standard deviation

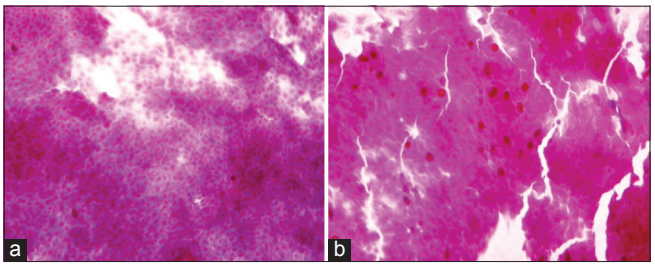

Figure 2. An impression cytology sample stained with PAS-Papanicolaou before (a) and after (b) treatment with cyclosporine A, note the increase in conjunctival goblet cell density (×200).

have revealed evidence for increased apoptosis in the conjunctival epithelium. This apoptosis also occurs in goblet cells resulting in a significant decrease in goblet cell density thus reducing mucin production and tear film stability.[13,16,17] A decrease in the total number of conjunctival goblet cells is the prominent histopathologic change in dry eye. Strong et al[18] showed that the use of topical cyclosporine 0.05% for 6 months decreased conjunctival epithelium apoptosis and increased goblet cell density in the bulbar and tarsal conjunctiva in experimental dry eye in mice. The effect of CsA 0.05% in decreasing apoptosis was believed to be due to its immune modulatory mechanisms. In 2002, Kunert et al showed a statistically significant effect of CsA 0.05% in tissue sections obtained from patients with keratoconjunctivitis sicca, in whom approximately a 200% increase in goblet cell density was detected after 6 months of therapy with CsA.[19]

The other histopathologic finding in samples obtained from patients with dry eye is squamous metaplasia, that is, defective epithelial cell differentiation from which goblet cells also originate, and this may alter goblet cell differentiation.[9,20,21] A clinical trial in 2008 evaluated the effectiveness of cyclosporine emulsion on conjunctival goblet cell density and showed that in dry eye patients cyclosporine emulsion for 12 weeks, increased goblet cell density; however artificial tears could not produce a similar effect.[13] In the other study, the efficacy of CSA 0.05% in dry eye disease was evaluated by Schirmer test, TBUT, OSDI and impression cytology. After 6 months of treatment, statistically significant improvement in these objective and subjective tests was seen and mean goblet cell density increased from 12.3 ± 8.7 to 33.0 ± 25.4/hpf.[12] In another study published in 2010 improvements in cytological grading of dry eye disease was observed after treatment with CsA 0.05%.[22] Consistent with previous studies, our data showed an improvement in symptoms and signs of dry eye disease and the density of bulbar conjunctival goblet cell increased from 23.3 ± 17.1 to 47.7 ± 16.1/hpf (approximately doubled) 3 months after treatment with CSA 0.05% twice daily. As the mechanism of action of cyclosporine is to modulate the immune response and decrease lymphocytic cell counts in the conjunctiva and lacrimal glands,[23] we hypothesize that treatment modalities targeting the inflammatory component of dry eye in a specific way are the most effective therapy for dry eye disease induced by mustard gas and should be considered as the main treatment strategy in these patients.

Although improvement in tests and parameters reflecting the severity of dry eye was statistically significant, clinical improvement was insignificant in some cases. The possible explanation is that our patients suffered from severe resistant dry eye disease which necessitates long term treatment and significant clinical improvement may be achieved by more prolonged treatment.

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