·Clinical Research·

Effect of topical 0.05% cyclosporine A on corneal endothelium in patients with dry eye disease

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Abstract

• AIM: To determine the effect of topical 0.05% cyclosporine A (CsA) on corneal endothelium in patients with dry eye disease.

• METHODS: Observational, prospective, case series study. Fifty-five eyes of 29 consecutive patients (9 males and 20 females; median age: 66.8 years, interquartile range: 61-73.2 years) with moderate-severe dry eye disease were evaluated. All patients were treated with topical 0.05% CsA ophthalmic emulsion twice a day in addition to lubricant eyedrops 5 times a day. The follow-up period was 12 months. Before treatment and at 3 and 12 months post-treatment central corneal specular microscopy was performed. The endothelial cell density (ECD), coefficient of variation of cell size (CoV), and percentage of hexagonal cells (Hex %) were analyzed.

• RESULTS: The median ECDs pre-treatment and at 3 and 12 months post-treatment were 2 352.5/mm^2 (interquartile range, 2 178-2548.5), 2 364/mm^2 (interquartile range, 2 174.25-2 657.5), and 2 366 cells/mm^2 (interquartile range, 2 174.75-2 539.75), respectively (P=0.927, one way ANOVA). The median CoVs pre-treatment and at 3 and 12 months post-treatment were 34.5 (interquartile range, 30-37), 35 (interquartile range, 30-38), and 34 (interquartile range, 30.75-38.25), respectively (P=0.7193, one way ANOVA). The median Hex % values pre-treatment and at 3 and 12 months post-treatment were 53 (interquartile range, 47-58), 54 (interquartile range, 45.75-59), and 50.5 (interquartile range, 45.75-58), respectively (P=0.824, one way ANOVA).

• CONCLUSION: Treatment of patients with dry eye disease for 12 months with topical 0.05% CsA does not seem to cause substantial changes on corneal endothelium.

• **KEYWORDS:** corneal endothelium; corneal toxicity; topical 0.05% cyclosporine A; dry eye disease; specular microscopy

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INTRODUCTION

Dry eye is a frequently encountered disorder of the tear film caused by tear deficiency or excessive evaporation of the tear film that causes damage to the interpalpebral ocular surface and is associated with symptoms of discomfort[1]. Decreased tear volume, increased osmolarity, disorder of cytokine balance, and increased matrix metalloproteinases can be seen in dry eye disease [2]. It has been demonstrated that inflammation and apoptosis might play a role in the development of dry eye. Because of chronic inflammation, conjunctival pathologic changes such as squamous metaplasia and goblet cell loss have been seen in cytological analysis of dry eye disease[3].

Traditional therapies for dry eye disease include artificial tears and punctal occlusion. More recently, cyclosporine A (CsA) 0.05% ophthalmic emulsion that addresses the underlying causes of the disease has been used to increase tear production in patients with chronic dry eye disease whose tear production is presumed to be suppressed owing to ocular inflammation. Clinical trials show that topical administration of CsA 0.05% significantly increases tear production, decreased concomitant artificial tear use, decreased ocular surface damage as assessed by fluorescein staining, and decreased symptoms such as burning, blurred vision, and dryness [4,5]. In addition, analysis of conjunctival epithelial biopsies from these patients showed decreases in molecular markers of immune activation and a large increase in conjunctival goblet cells, the source of soluble mucins in the tear film[3,4,6-10].

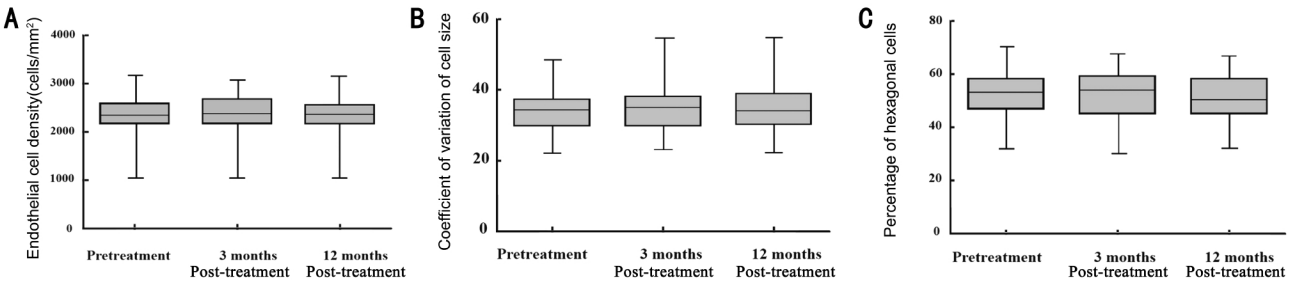

Figure 1 The box plots (median value with interquartile range) show no significant (one way ANOVA) difference between the pre-treatment and post-treatment A: Endothelial cell density values, P=0.927; B: Coefficient of variation of cell size values, P=0.719; C: Hexagonal cell measurements, P=0.824.

After topical application, CsA accumulates at the ocular surface and cornea (epithelium, stroma and endothelium), and very little drug penetrates to intraocular tissues [11]. Therefore, we believed to be appropriate to investigate its toxic reactions on corneal endothelium, which remains one of the more vulnerable cells to the toxicity of drugs in the eye[12-14]. In the current study, using specular microscopy with morphometric analysis, a sensitive indicator of endothelial cell function [15], we prospectively analyzed the *in vivo* corneal endothelium toxicity after topical 0.05% CsA administration to treat dry eye disease.

SUBJECTS AND METHODS

Subjects Fifty-five eyes of 29 consecutive patients with moderate to severe dry eye disease were recruited for this observational prospective case series study. The study protocol was approved by the Ethics Committee of the University Hospital Principe de Asturias and adhered to the tenets of the Declaration of Helsinki. All participants provided informed consent. Moderate to severe dry eye disease was defined by symptoms of ocular irritation and fluorescein staining pattern with Schirmer test reading (without anesthesia) of ≤5mm/5min or tear film break-up time (BUT) less than 6s. The exclusion criteria were systemic medications that inhibit tear production or eyedrops other than unpreserved tears; previous punctual plug; history of contact lenses use; previous ocular surgery or trauma; and ocular and systemic diseases, such as Fuch's dystrophy, diabetes and connective tissue disorders that could alter the morphology of the corneal endothelium.

All patients were treated with topical 0.05% CsA ophthalmic emulsion twice a day in addition to lubricant eyedrops (Acuolens, AlconCusí, Barcelona, Spain) 5 times a day. At the baseline visit, after a complete ophthalmological examination and before administration of treatment, specular microscopy with morphometric analysis was performed, and repeated at 3 and 12 months.

Methods

Endothelial cell analysis Noncontact specular microscopy was performed on the central cornea using the specular microscope SP-3000P (Topcon, Corp, Tokyo, Japan). A masked observer (A.M.S.) obtained 3 corneal endothelial images and the average of the measurements was used to calculate the values analyzed for each patient. The specular microscope automatically evaluated the endothelial cell density (ECD), the coefficient of variation of the cell size (CoV) (an objective measure of polymegathism), and the percentage of the hexagonal cells (Hex%) (index of pleomorphism). The same masked observer performed manual endothelial cell analyses. At least 75 well-defined cell borders were counted manually in a defined square of the picture, which corresponded to 0.03mm^2 of the actual cornea.

Statistical Analysis All data are expressed as the median and interquartile range. The differences between the pre-treatment and post-treatment measurements were evaluated by one-way analysis of variance with the Tukey multiple comparison post test. Statistical analysis was performed using GraphPad InStat version 3.00 for Windows 95, GraphPad Software (San Diego, CA, USA). The study design had a power of 90% to detect a change in the endothelial cell count of 10% or more, with the significance level set at 0.05.

RESULTS

The study group included 9 male and 20 female. The median patient age was 66.8 years (interquartile range, 61-73.2 years). All patients completed 12 months of follow-up. No systemic adverse effects of topical CsA treatment were recorded during this study. The median Schirmer score at baseline was 3mm (interquartile range, 2mm-4mm) and the median BUT score was 4s (interquartile range, 3s-5s).

ECD and morphology All measurements were performed before topical 0.05% CsA treatment and at 3 and 12 months post-treatment. The median ECDs at those time points were 2 352.5/mm^2 (interquartile range, 2 178-2 548.5), 2 364/mm^2 (interquartile range, 2 174.25-2 657.5), and 2 366/mm^2 (interquartile range, 2 174.75-2 539.75) (Figure 1A). The median CoVs were 34.5 (interquartile range, 30-37), 35 (interquartile range, 30-38), and 34 (interquartile range, 30.75-38.25) (Figure 1B). The median Hex % values were 53 (interquartile range, 47-58), 54 (interquartile range, 45.75-59), and 50.5 (interquartile range, 45.75-58) (Figure 1C). There

were no statistical variations in these parameters after topical 0.05% CsA administration (ECD, P=0.927; CoV, P=0.719; Hex %, P=0.824).

DISCUSSION

Our results indicated that topical 0.05% CsA ophthalmic emulsion twice a day in dry eye disease did not cause a substantial change in the corneal ECD, CoV, or Hex% values compared with the pre-treatment values. A regular hexagonal endothelial cell pattern provides a stable covering, and any alteration in this hexagonal pattern reflects loss of endothelial cell function. A previous study [16], also in dry eye, reported that topical CsA 0.05% did not cause significant changes on corneal morphology and function as corneal thickness, topographical findings, ECD, and corneal biomechanical parameters. In that study, the hexagonal cells and polymorphism were not evaluated. However, it is well known that the ECD and corneal thickness measurements are not reliable indicators of morphologic changes and that CoV and hexagonal cell arrangements are more important. In the current study, all ECD, CoV, and Hex % values were evaluated and no significant differences were found in any quantitative endothelial morphometric parameters investigated, suggesting the absence of long-term corneal endothelium changes.

It has been demonstrated that inflammation has a major role in dry eye disease pathogenesis. This inflammation consists of inflammatory cell infiltration of the ocular surface, activation of the ocular surface epithelium with increased expression of adhesion molecules and inflammatory cytokines, and increased concentrations of inflammatory cytokines in the tear fluid[17,18]. It is already known that CsA is an effective immunomodulator and anti-inflammatory agent that has been used for systemic therapy for many years and more recently has become available for topical use in dry eye disease treatment. CsA prevents synthesis and secretion of several proinflammatory cytokines, such as tumor necrosis factor α and interleukin 6, and has beneficial effects on the underlying inflammatory pathology of dry eye syndrome[4,6]. It was reported that there was a significant decrease in the immune activation markers HLA-DR, CD11a, and interleukin 6 levels after 6 months of treatment with 0.05% CsA [7,8]. Furthermore, several studies showed a significant improvement in aqueous tear production and increase in goblet cell density compared to other therapies in patients with dry eye disease[3,9,10,19,20]. Strong *et al*[21] also showed that topical CsA significantly reduced conjunctival epithelial apoptosis and goblet cell loss in experimental murine keratoconjunctivitis sicca. More recently, De Paiva *et al*[22] have demonstrated that CsA treatment increased interleukin 13 that has an essential role in homeostatic maintenance of conjunctival goblet cells.

CsA accumulates at the ocular surface and cornea (epithelium, stroma and endothelium) after topical application and very little drug penetrates to intraocular tissues [11]. In this sense, the safety of CsA ophthalmic emulsions was evaluated by ocular studies in laboratory animals and long-term clinical trials [4,23-25]. However, few experimental studies of the safety of CsA on ocular cells and tissues have been reported. An *in vitro* study[26] demonstrated that cultured human corneal endothelial and stromal cells exposed to CsA exhibited no adverse effects and only minor effects on DNA synthesis. More recently, Garweg *et al*[27] evaluated the chronic toxicity of CsA by monitoring the proliferation and viability of subconfluent cultures of human corneal endothelial cells after continuous exposure to the drug for 7 days. At concentrations below 5μg/mL, they reported that both cell proliferation and cell metabolism showed no substantial decline. Ours findings agree with the reports of those studies that described the effects of CsA on corneal endothelial cells and no clinical evidence of endothelial damage has been noted.

This study had one limitation. A control group might have helped to establish the absolute normality of the corneal endothelium of our patients at baseline. Brooks *et al*[28] reported that in chronic cases of superficial keratopathy of varied etiology, including dry eye syndrome, changes in endothelial morphology may occur, with pleomorphism and polymegatism of the endothelial cells. In contrast, Erdélyi *et al*[29], using confocal microscopy, not detected endothelial changes in aqueous-deficient dry eye. In this sense, all our patients both at baseline and in follow-up period showed a normal corneal endothelium without evidence of pleomorphism or polymegethism.

Corneal endothelial cell function has been not tested in humans after CsA treatment. In the current study, using specular microscopy, we have demonstrated that the treatment of patients with dry eye for 12 months with topical 0.05% CsA does not seem to cause substantial changes on corneal endothelium. These data are consistent with the clinical observation that the immunomodulatory drug CsA may be a suitable candidate for the control of dry eye disease.

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