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Comparison of therapeutic effects of 0.05% Cyclosporine A versus 0.1% Fluorometholone in Chinese patients with mild dry eye unresponsive to artificial tears: a randomized control study

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Abstract

Background To assess and compare the therapeutic outcomes of 0.05% Cyclosporine A (CsA) ophthalmic solution versus 0.1% Fluorometholone (FML) eyedrops in Chinese patients with mild dry eye disease (DED) unresponsive to conventional artificial tears (AT).

Methods A total of 43 patients with mild DED, who have failed to respond to conventional AT therapy for over 3 months, were randomly assigned to receive either 0.05% CsA or 0.1% FML twice daily for 6-months. In addition, all the patients were instructed to use 0.1% SH 4 times a day as supplementary therapy. Dry eye examination, including Ocular Surface Disease Index (OSDI) questionnaire, non-invasive tear break-up time (NIBUT), Schirmer scores, corneal fluorescein staining (CFS) scores, and conjunctival goblet cell (CGC) density, intraocular pressure (IOP), Best corrected visual acuity (BCVA) was conducted at baseline and then evaluated at 1, 3, and 6 months after treatment. Corneal endothelial cell density, corneal dendritic cells (DCs) and nerves were assessed by in vivo confocal microscopy at baseline and 6 months after treatment.

Results At 3 and 6 months after treatment, OSDI scores in the 0.05% CsA group showed more improvement than those in the 0.1% FML group. CFS was significantly lower and Schirmer scores were significantly higher in 0.05% CsA group compared with 0.1% FML group. NIBUT improved significantly in both groups, with greater improvement in the 0.05% CsA group at the 1-, 3-, and 6-month visits. Throughout the duration of the study, the 0.1% FML group exhibited no notable enhancement in CGC density. Conversely, a substantial elevation in CGC density was observed in the 0.05% CsA group. After 6 months of treatment, significantly reduced corneal DC density and area were obtained in 0.05% CsA group as compared to 0.1% FML group, while there were no significant changes in cornea nerve fiber density, cornea nerve fiber length and cornea nerve fiber width in both groups. Additionally, after 6 months

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of treatment, neither group showed any statistically significant changes in IOP, BCVA or in corneal endothelial cell density.

Conclusion The administration of 0.05% CsA proved effective in managing mild DED, offering a supplementary advantage in improving Schirmer scores, restoring CGC density and reducing corneal DC density compared to 0.1% FML eyedrops. Consequently, 0.05% CsA eyedrops are recommended as a safe and efficacious therapeutic alternative for patients with mild DED who fail to respond to conventional tear substitutes therapy.

Clinical trial registration number Chinese Clinical Trial Registry, ChiCTR2200066441, Registered 06 December 2022-Retrospectively registered.

Keywords 0.05% cyclosporine A, 0.1% fluorometholone, Mild dry eye disease, Conjunctival goblet cells, Dendritic cells, Corneal nerve

Background

Dry eye is the most prevalent ophthalmic disease, affecting millions worldwide with increasing frequency [1, 2]. It is recognized as a multifactorial disease involving the ocular surface and tear film, characterized by variations in signs and symptoms [3, 4]. As the pathophysiology of dry eye disease (DED) has been established through numerous studies, ocular surface inflammation is recognized as the primary cause of DED. Multiple factors trigger the production of inflammatory mediators on the ocular surface, leading to potential damage to the cornea, conjunctiva, and even loss of vision [5, 6]. Therefore, controlling ocular surface inflammation as early as possible and mitigating potential damage is crucial in treating DED.

Cyclosporine A (CsA) is an established systemic immunosuppressant with anti-inflammatory properties that prevents T-cell activation and inflammatory cytokines production [7, 8]. In 2002, 0.05% CsA ophthalmic emulsion (Restasis, Allergan, Irvine) was approved by the U.S. Food and Drug Administration (FDA) for the treatment of DED. Numerous reports have demonstrated that 0.05% CsA is safe and effective for managing moderate to severe DED and has a beneficial effect on the underlying inflammatory pathological conditions of DED [9, 10]. However, the therapeutic effects of 0.05% CsA in the Chinese population and its role in mild DED treatment have not been fully studied.

Artificial tears (AT) as a first-line therapy to improve DED symptoms have traditionally been used in the treatment of all stages of the disease. However, even in patients with mild DED, tear substitutes are not effective for all [1, 11]. Recently, 0.05% CsA ophthalmic emulsion (Cycloome; Shenyang Xingqi Pharmaceutical Co. Ltd., China) has been approved for DED treatment in China. The objective of the study described here was to explore the therapeutic effect of 0.05% CsA eye drops in mild DED patients unresponsive to conventional AT. Our objective is to investigate the therapeutic impact of 0.05% CsA in the Chinese population and generate additional clinical evidence to endorse the utilization of 0.05% CsA in individuals with mild DED.

Materials and methods

Participants

This prospective study includes 43 eyes of 43 patients who were diagnosed as mild DED at Tianjin Medical University Eye Hospital from August 2021 to December 2022. All participants were initially diagnosed with DED in accordance with the diagnostic criteria of Chinese dry eye experts: examination and diagnosis (2020) [12]. Subsequently, mild DED patients were classified based on the consensus of Chinese dry eye experts: definition and classification (2020) [13]: slit lamp microscope examination showed no obvious signs of ocular surface injury (corneal fluorescein staining less than 5 points), and the patients were nonresponsive to AT treatment for more than 3 months. The study adhered to the Helsinki Declaration and was approved by the Ethics Committee of Tianjin Medical University Eye Hospital (Ethical batch number: 2021KY-17). Written informed consent was obtained from all subjects. Additional patient inclusion and exclusion criteria are presented in Table 1.

Study protocol

Forty-three patients were randomly divided into two groups and instructed to use topical 0.05% CsA (Cycloome®) or 0.1% Fluorometholone (FML, Flumetholon®, Santen, Japan) respectively, both twice a day for 6 months. In addition, each patient was instructed to use 0.1% SH (Hialid®, Santen, Japan) eye drops at a frequency of 4 times per day, and to reduce the potential for washing out the study drug, they were required to maintain a minimum interval of 1 h between the use of 0.1% SH and either CsA or FML. Study follow-up visits occurred on day-1(baseline, visit1), 1-month (visit 2), 3-month (visit 3), and 6-month (visit 4). All patients underwent a standardized dry eye examination, which included Ocular Surface Disease Index (OSDI) scores, non-invasive tear break-up time (NIBUT), Schirmer I test, corneal fluorescein staining (CFS) scores, intraocular pressure

Table 1 Patient inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Between 18 and 70 years of age	Use of topical or systemic steroids or IS within the past 1 mo
Patient in general good health	Allergy or sensitivity to CSA or FML
Daily life vision ≥ 0.10 (Decimal)	Use of other ophthalmic drugs
Diagnosed with mild dry eye according to the consensus of Chinese dry eye experts: definition and classification (2020)	Contact lens wear within the past 1 mo
	Intraocular surgery within 6 mo
Nonresponsive to AT treatment>3 mo	Ocular surface infection/ inflammation
Informed consent signed	Pregnant/breast feeding women

Chinese dry eye experts: definition and classification (2020): diagnosed with mild dry eye (corneal fluorescein staining less than 5 point)

AT, artificial tears; mo, months; IS, immunosuppressants; CsA, Cyclosporine A; FML, Fluorometholone

(IOP), Best corrected visual acuity (BCVA, Decimal notation), and conjunctival goblet cell (CGC) density at baseline and at 1, 3, and 6 months after treatment. Corneal endothelial cell density, corneal dendritic cells (DCs) and nerves were assessed by in vivo confocal microscopy (IVCM) at baseline and at 6 months of treatment. As a safety measure, IOP was measured using a non-contact tonometer (CT-1, Topcon, Japan). The protocol required that patients with IOP elevation above 25mmHg or below 5mmHg be withdrawn from the study and stop using all study medications (Fig. 1).

Dry eye signs and symptoms

The NIBUT was measured by the Keratograph 5 M (K5M, Oculus, Germany) as described previously [14]. The time between the last blink and the first sign of distortion in the ring pattern was recorded as NIBUT, and the average of 3 repeated measurements was recorded. The Schirmer I test without anesthesia was to measure the secretory function of the lacrimal gland. The Schirmer strip (Tianjin Jingming New Technological Development Co. Ltd., China) was gently placed in the lateral one-third of the lower eyelid, and the wetted length of the strip was recorded after 5 min. The CFS was observed with a slit lamp biomicroscope with fluorescein staining and was scored on a scale of 0–12 based on the sum of the four quadrant scores of the cornea [15]. The degree of the staining in each quadrant was graded from 0 to 3 (0, no staining; 1, for 1–30 punctate staining; 2, more than 30 punctate staining but not fused; and 3, corneal punctate coloration fusion, filamentous and ulcer).

The OSDI questionnaire was employed to evaluate the discomfort symptoms associated with DED [16]. The OSDI scoring scale ranges from 0 to 100, with higher scores indicating more severe symptoms of DED. All patients are required to complete the questionnaire, and the total OSDI scores are calculated using the following formula: OSDI = [(total sum of scores for all questions answered) × 100] / [(total number of questions answered) × 4] [17].

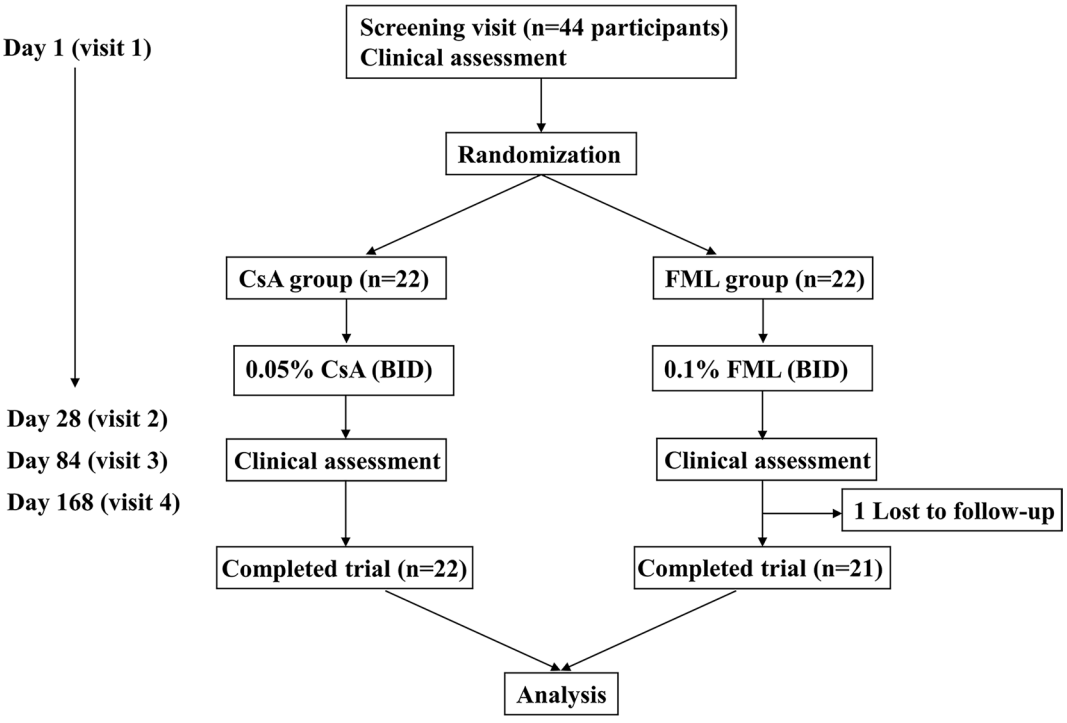

Fig. 1 Flow diagram of study progress in the 0.05% CsA and 0.1% FML groups. CsA, Cyclosporine A; FML, Fluorometholone; BID: twice a day; QID: four times a day

CGC density

After the ophthalmological examination, impression cytology was performed at baseline, 1 month, 3 months, and 6 months. CGCs were harvested using the cellulose acetate membrane by placing it onto the superior temporal bulbar conjunctiva for 5 s under local anesthesia with 0.5% proparacaine hydrochloride (Alcaine; Alcon, TX, USA). The samples were fixed with paraformaldehyde solution, dyed with periodate Schiff (PAS), and observed under a light microscope. The CGC density was calculated by counting 5 randomly chosen areas and was presented as cells/mm^2.

IVCM

The tissue structure of each layer of the central cornea of both eyes was scanned by IVCM (HRT-3, Heidelberg Engineering GmbH, Heidelberg, Germany). During the examination, the subjects were asked to fixate on a target under local anesthesia, and the examiner gradually moved the machine so that the IVCM could examine the cornea at different depths. The two-dimensional images have a definition of 384×384 pixels with an area of 400 μm × 400 μm. Each eye randomly selected 5 single-layer images with the best focus and contrast for analysis. The DC density and area were measured by ImageJ software. Cornea nerve fiber density (CNFD), cornea nerve fiber length (CNFL) and cornea nerve fiber width (CNFW) were analyzed by ACCMetrics software [18]. The corneal endothelial cells were manually counted and recorded utilizing the cell counting tool within the IVCM system. For each individual eye, two IVCM images depicting the central corneal endothelium were assessed, and the average value were recorded and presented as n/mm^2.

Statistical analysis

The data were analyzed by GraphPad Prism 9.0 software. The normality of the measurement data was determined using Kolmogorov-Smirnov test and expressed as Mean±SD. The baseline data of the two groups were compared by independent sample t-test or chi-square test. The drug efficacy between the two groups was compared by Repeated Measures Analysis of Variance (ANOVA) and Student's t-test. All statistical significance was set at $p<0.05$. We performed a statistical power analysis for both the OSDI score and NIBUT at visit 4 in the 0.05% CsA and 0.1% FML groups. The power (1-β) was >0.80 at the level of α=0.05, and the sample size was sufficient.

Results

Patient demographics

A total of 44 patients participated in our study, with 43 individuals (43 eyes) completing the study (dropout rate: 2.27%) and the patient demographics are shown in Table 2. There were no significant differences in terms of sex ratio (male/female: 4/18 vs. 6/15, $p=0.4876$) and age (53.41±11.04 vs. 52.90±11.35 years, $p=0.8833$) between the two groups.

Table 2 Demographics of study population

Characteristics	0.1% FML	0.05% CsA	*p*
Number	21	22	-
Sex (M/F)	6/15	4/18	0.4876[a]
Age (years)	52.9±11.35	53.41±11.04	0.8833[b]

CsA, Cyclosporin A; FML, Fluorometholone; M, Male; F, Female

a: Chi-square test, b: Independent sample t-test

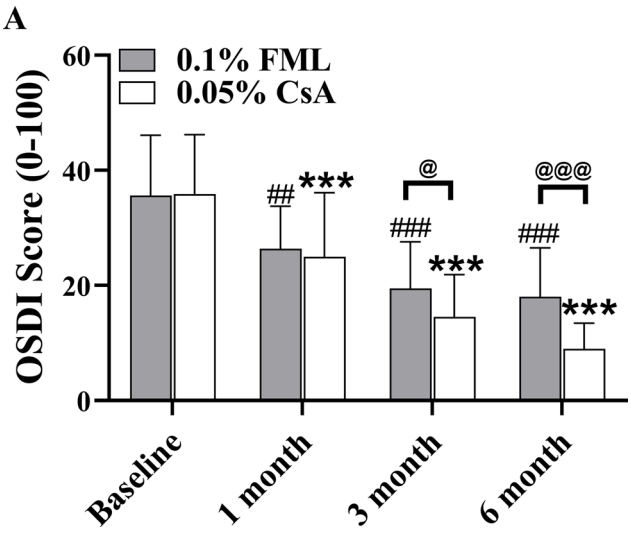

Fig. 2 Comparison of OSDI scores between 0.05% CsA group and 0.1% FML group. *0.05% CsA treatment compared with baseline, ***$p<0.001$. # 0.1% FML treatment compared with baseline, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$. @ 0.05% CsA group versus 0.1% FML group, $^{@}p<0.05$, $^{@@@}p<0.001$

Outcome data and main results

Dry eye symptoms

Subjective symptoms of DED were evaluated by the OSDI questionnaire, and the mean OSDI scores in two groups at each follow-up time point were shown in Fig. 2. There was no significant difference in the OSDI scores between the two groups at baseline ($p=0.9307$). After treatment with 0.05% CsA or 0.1% FML, statistically significant improvements of OSDI scores were observed in both group at 1, 3, and 6 months ($p<0.01$ for all). In addition, our data showed significantly lower OSDI scores in the 0.05% CsA group compared with those in the 0.1% FML group at 3 and 6 months after treatment ($p=0.0425$, $p=0.0001$, respectively).

CFS, schirmer scores and NIBUT

CFS, Schirmer scores and NIBUT are commonly used clinical parameters for DED. As shown in Fig. 3A, there was no significant difference in CFS scores between the

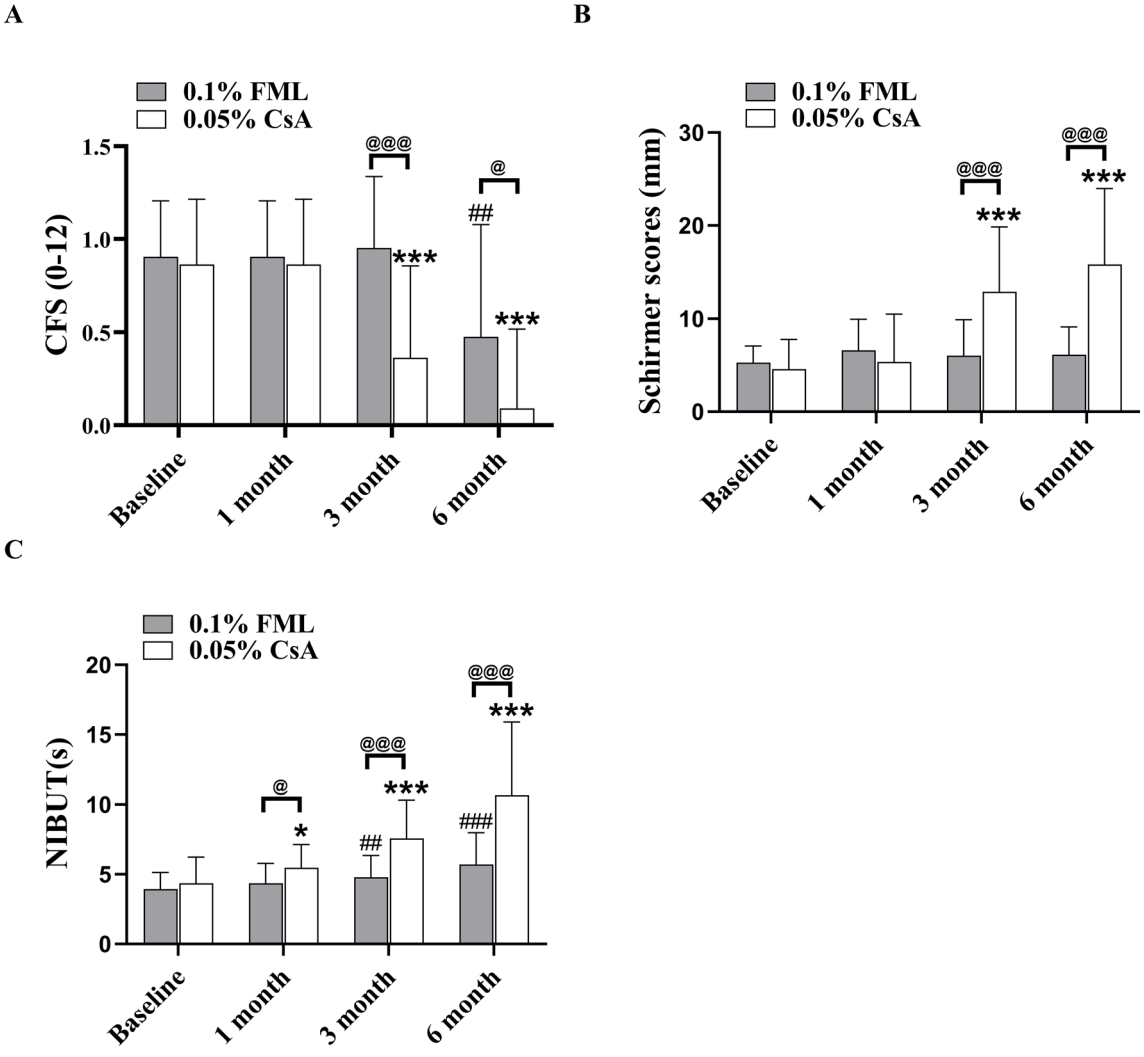

Fig. 3 Changes in clinical parameters of dry eye disease after treatment with 0.05% CsA or 0.1% FML. (**A**) CFS, corneal fluorescein staining scores. (**B**) Schirmer scores. (**C**) NIBUT, non-invasive tear break-up time. *0.05% CsA treatment compared with baseline, $^{*}p<0.05$, $^{***}p<0.001$. # 0.1% FML treatment compared with baseline, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$. @ 0.05% CsA group versus 0.1% FML group, $^{@}p<0.05$, $^{@@@}p<0.001$

two groups at baseline ($p=0.6818$) and the second visit (1 month, $p=0.6818$). The CFS scores were significantly improved at 6 months after treatment with 0.1% FML ($p=0.0037$), while the 0.05% CsA group exhibited more significant improvement in CFS at both 3 ($p<0.0001$) and 6 months ($p=0.0211$) compared to the 0.1% FML group.

The changes in Schirmer scores in both groups are detailed in Fig. 3B. There was no statistically significant improvement in Schirmer score from baseline in the 0.1% FML group at any of the follow-up visits ($p>0.05$ for all). However, patients in the 0.05% CsA group exhibited significantly higher Schirmer scores at the 3- and 6- month treatment periods ($p<0.001$ for all).

As summarized in Fig. 3C, both groups demonstrated significant improvement in NIBUT, with continuous improvements observed between months 1 and 6 in the 0.05% CsA group ($p<0.05$ for all). Statistically significant improvement in NIBUT was observed in the 0.05% CsA group compared to the 0.1% FML group at all follow-up visits ($p<0.05$ for all).

CGC density

Since the loss of CGC is a hallmark of DED [19], we examined the density of CGC using conjunctival imprint cytology staining. At baseline, the CGC density ranged between 125.40±57.59 and 143.52±67.09 cells/mm^2, with no statistically significant difference among the groups ($p=0.3485$). At all follow-up visits, the CGC density in the 0.05% CsA group showed statistically significant improvement compared to baseline ($p<0.01$ for all), while there was no significant change in the CGC density of the 0.1% FML group ($p>0.05$ for all) (Fig. 4A). Representative conjunctival imprint cytology staining images for both groups are shown in Fig. 4B.

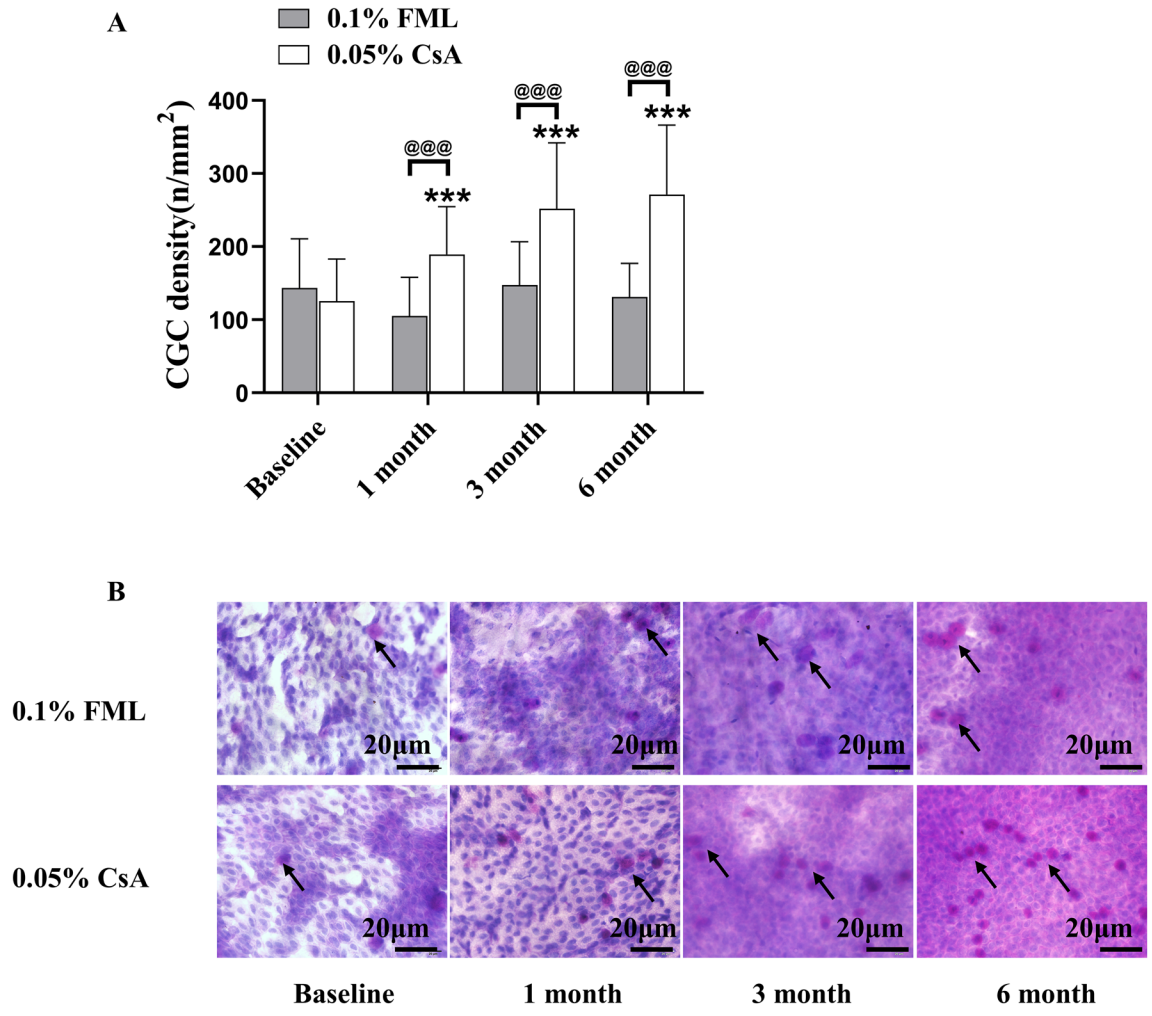

Fig. 4 Changes in CGC density after treatment with 0.05% CsA or 0.1% FML (**A**) CGC density, conjunctival goblet cell density. *0.05% CsA treatment compared with baseline, ***$p<0.001$. @ 0.05% CsA group versus 0.1% FML group, $^{@@@}p<0.001$. (**B**) Representative images (PAS staining) of conjunctival goblet cells in both groups (black arrows)

DC density and area

Given that the increase in corneal DC density and area is crucial for the pathogenesis of DED, we employed IVCM to measure the density and area of corneal DCs. As shown in Fig. 5A-B, there was no statistically significant difference in DC density ($p=0.9550$) and area ($p=0.9558$) between the two groups at baseline. After 6 months of treatment, the 0.05% CsA group exhibited a significant reduction in both DC density ($p=0.0003$) and area ($p<0.0001$) compared to the 0.1% FML group. The representative IVCM images in two groups were shown in Fig. 5C.

CFND, CNFL and CNFW

At baseline, the mean CNFD, CNFL and CNFW were 19.78±2.58 n/mm^2, 13.50±2.11, and 0.02±0.001 mm/mm^2 respectively in 0.05% CsA group and 19.89±3.26 n/mm^2, 13.48±2.20, and 0.02±0.001 mm/mm^2 respectively in 0.1% FML group, with no statistically significant differences among the groups ($p=0.9038$, $p=0.9780$, $p=0.9133$, respectively). After scheduled treatment with 0.05% CsA or 0.1% FML for 6 months, there were no significant change in CNFD, CNFL and CNFW in both groups ($p=0.2315$, $p=0.6138$, $p=0.2904$, respectively) (Table 3).

Safety outcomes

IOP and BCVA were measured at each study visit, and corneal endothelial cells were assessed at baseline and the end of the study. Throughout the course of the study, there were no significant changes in IOP and BCVA in either the 0.05% CsA or 0.1% FML treatment group. There was no statistically significant difference in the comparison of corneal endothelial cells between the two groups before and after treatment (Table 4). Throughout the research period, there were no ocular adverse events

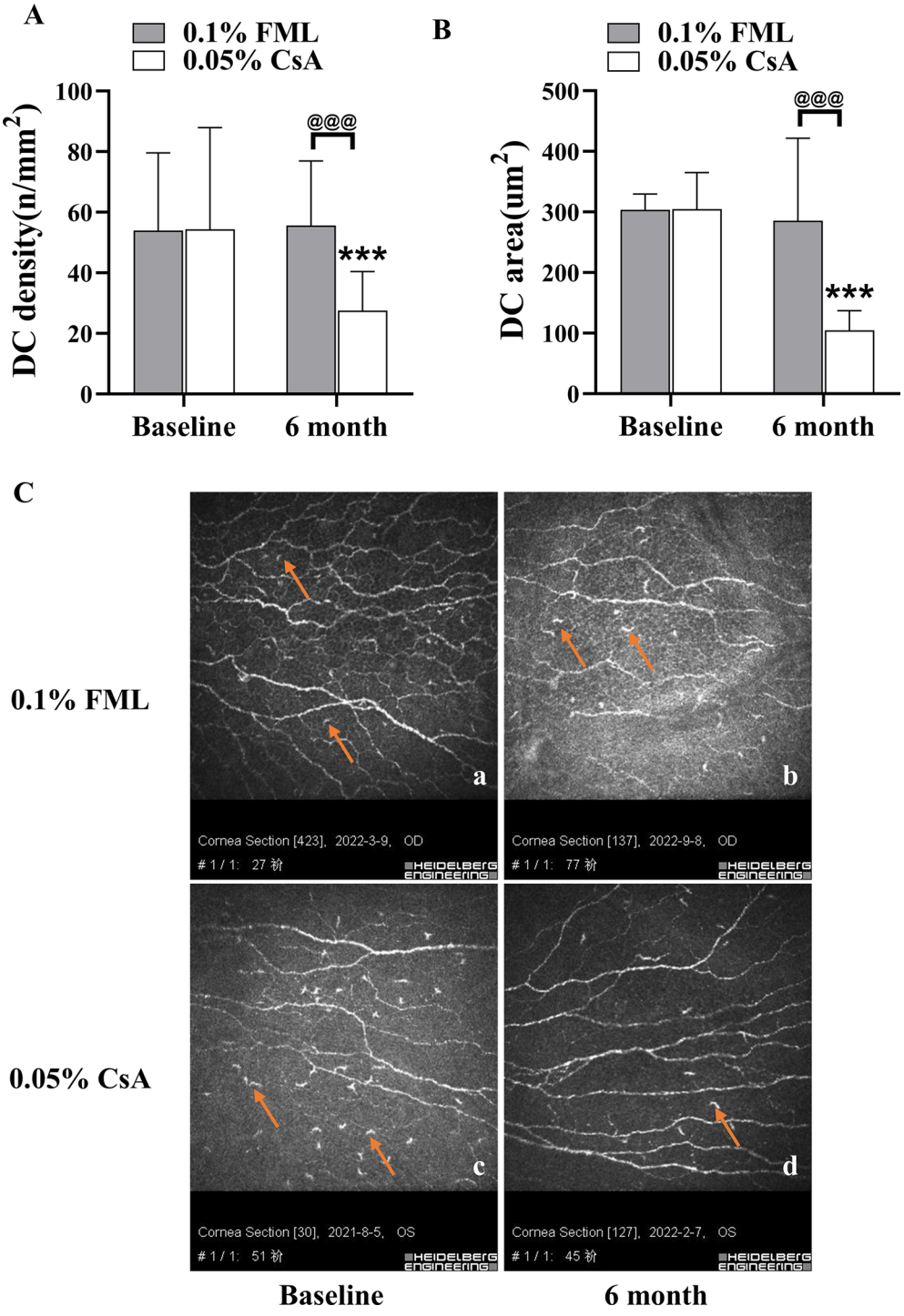

Fig. 5 Changes in DC density and area after treatment with 0.05% CsA or 0.1% FML. (**A**-**B**) *0.05% CsA treatment compared with baseline, ***$p < 0.001$. @ 0.05% CsA group versus 0.1% FML group, $^{@@@}p < 0.001$. (**C**) DC density observed on in vivo confocal imaging (orange arrows)

Table 3 Comparison of CFND, CNFL and CNFW for participants from both groups (Mean ± SD)

Group	CNFD(n/mm^2)		CNFL(mm/mm^2)		CNFW(mm/mm^2)	
	Baseline	6 month	Baseline	6 month	Baseline	6 month
0.1% FML	19.89 ± 3.26	19.86 ± 3.73	13.48 ± 2.20	13.52 ± 2.81	0.02 ± 0.001	0.02 ± 0.002
0.05% CsA	19.78 ± 2.58	21.58 ± 5.37	13.50 ± 2.11	13.93 ± 2.51	0.02 ± 0.001	0.02 ± 0.001
p	0.9038	0.2315	0.9780	0.6138	0.9133	0.2904

CsA, Cyclosporin A; FML, Fluorometholone; CNFD, Cornea nerve fiber density

CNFL, Cornea nerve fiber length; CNFW, Cornea nerve fiber width

Table 4 Safety outcomes (Mean ± SD)

Treatment	Visit 1	Visit 2	Visit 3	Visit 4	*p*
IOP (mm/Hg)					
0.05% CsA	15.17 ± 3.19	15.32 ± 2.27	15.32 ± 2.91	15.36 ± 2.82	0.9966
1% FML	14.91 ± 2.76	15.39 ± 2.92	15.53 ± 1.87	15.68 ± 2.66	0.7887
BCVA (Decimal)					
0.05% CsA	0.95 ± 0.13	0.95 ± 0.13	0.96 ± 0.12	0.96 ± 0.10	0.9138
1% FML	0.91 ± 0.12	0.98 ± 0.06	0.93 ± 0.12	0.92 ± 0.13	0.2213
Corneal endothelial cells (n/mm^2)					
0.05% CsA	2563.82 ± 325.06	-	-	2546.00 ± 327.32	0.8571
1% FML	2589.05 ± 242.43	-	-	2550.05 ± 233.90	0.5987

IOP, intraocular pressure (IOP); BCVA, Best corrected visual acuity; CsA, Cyclosporin A; FML, Fluorometholone

reported in either treatment group, such as redness, discomfort, soreness, pain, or tearing.

Discussion

Anti-inflammatory therapies such as topical steroids or CsA are often recommended for the treatment of moderate to severe DED [20, 21]. With the in-depth investigation of the pathogenesis of DED, more and more clinicians have realized the importance of anti-inflammatory treatment in the early stage of the DED [22–24]. A South Korean study [25] showed that in the real world, topical anti-inflammatory agents were commonly used for the treatment of grade 1 dry eye patients, with topical steroids used in 46.9% of cases and 0.05% CsA used in 41.8%. Notably, FML was the most used topical steroid, accounting for 90.8% of patients used steroids [20, 25]. However, the safety and effectiveness of topical CsA and topical steroids in the treatment of mild DED, as well as the duration of anti-inflammatory therapy, still need to be further explored.

Prior studies have demonstrated the benefits of topical CsA in alleviating the signs and symptoms of DED, and CsA formulations with concentrations of 0.05% and 0.1% are regarded as the most suitable for treating DED, as no further benefits have been observed with increased concentrations [26]. Upon comparison with 0.05% CsA, 0.1% CsA has proven to be more efficacious in patients experiencing severe dry eye conditions, particularly those with Sjögren's syndrome-related dry eye or those unresponsive to the topical 0.05% regimen [21, 27]. Over the years, researchers have devoted considerable efforts to enhancing ocular drug delivery systems for CsA, addressing challenges associated with its limited solubility, high molecular weight, and hydrophobic nature [28]. Currently, marketed CsA ophthalmic solutions usually utilize castor oil, corn oil, ethanol, and other solubilizers and surfactants in their prescription composition. Nevertheless, despite their effectiveness in dissolving CsA, these auxiliaries pose certain safety concerns and irritation issues [28, 29]. Recently, a water-free 0.1% CsA solution has proven to be effective in DED treatment. However, it remains in the clinical research stage, and its safety and efficacy are currently undergoing further investigation [30, 31]. In the present study, we employed Cycloome®, the first 0.05% CsA ophthalmic emulsion approved for treating DED in China. This formulation achieved a transformation from an emulsion to a nano-microemulsion type, exhibiting a more uniform particle size and a more comfortable ocular experience compared to Restasis®, the 0.05% CsA ophthalmic emulsion approved by the FDA in 2002. Our results showed that, compared to baseline, Cycloome® was effective in reducing corneal staining and improving ocular dryness, which was consistent with the previous study [32].

Affected by factors such as video terminals and environmental pollution, the number of patients with DED, especially those with mild symptoms, has increased sharply [33, 34]. The 0.05% CsA ophthalmic solution has proven effective in treating moderate to severe dry eye, yet only a limited number of studies have included patients with mild DED. Yüksel et al. [35] reported that 0.05% CsA was effective in improving Schirmer scores, BUT, and OSDI scores in patients with mild DED. Nevertheless, their findings were limited to a small cohort of 12

patients. Perry et al. [36] enrolled 62 patients with mild DED and found that, in comparison to those with moderate to severe DED, topical CsA had the greatest symptomatic benefit in patients with mild DED. Nevertheless, they did not compare the therapeutic effects of topical CsA with topical steroids, the two most commonly used anti-inflammatory treatments for mild DED in the real world. In the present study, we found that 0.05% CsA was effective in the treatment of mild DED and had a significant advantage in improving Schirmer scores and BUT compared to 0.1% FML eye drops, demonstrating that 0.05% CsA could effectively increase tear secretion and improve tear film stability in mild DED patients. The therapeutic effect of 0.05% CsA was most beneficial after 3 months of treatment, which is consistent with previous findings [36], indicating that the longer the treatment duration, the better the improvement, and the more benefits patients will gain.

CGCs play a crucial role in maintaining the stability of tear film and serve as sensitive indicators of DED [37, 38]. While topical CsA has been verified to effectively increase the number of CGCs in DED patients [10, 39], its impact on CGCs in patients with mild DED remains sparsely reported. Our previous study indicated that the decrease in CGCs may be an indicator of mild DED patients who are unresponsive to AT therapy (unpublished data). In our current study, we observed that the density of CGCs significantly increased in patients with mild DED after 1 month of treatment with 0.05% CsA, whereas the 0.1% FML group exhibited no notable enhancement in CGCs density throughout the study. Given that the loss of CGCs has been demonstrated to be positively correlated with inflammatory response in DED [37, 40], the protective effect of CsA on CGCs in patients with mild DED may be conducive to restoring ocular surface immune homeostasis and delaying disease progression.

IVCM is a powerful tool for detecting early immune alterations and potentially cellular inflammation on the ocular surface [41, 42]. Using IVCM, we observed a notable reduction in the density and area of corneal DCs among patients with mild DED following treatment with 0.05% CsA. Previous studies have demonstrated that corneal DC density was correlated with DED severity, with significant increase in DC density observed even in patients with grade 1 DED [42]. Our findings indicated that early treatment with CsA eye drops may lead to a better treatment response. The decrease in DC density promoted by CsA possibly due to its effect in breaking the vicious cycle of DED inflammation. Levy et al. reported that topical CsA significantly increased the subbasal corneal nerves density in Sjögren syndrome DED patients [43]. However, we did not observe any significant changes in CNFD, CNFL, or CNFW among patients treated with CsA. The absence of significant pathological changes in the subcorneal nerves during the early stages of DED may explain the discrepancy among these studies.

A limitation of this study is the relatively small sample size. Future studies including larger populations can provide more information regarding the effect of 0.05% CsA on mild DED. In addition, more objective indicators of ocular surface inflammation such as tear cytokine detection will better explain the mechanism of CsA treatment for mild DED.

Conclusions

To conclude, in patients with mild DED, treatment with 0.05% CsA for 6 months led to a clinically significant improvement in the signs and symptoms of DED, an increase in CGCs density, and a decrease in DC density compared with 0.1% FML. Therefore, early treatment with CsA eye drops may interrupt the vicious cycle of inflammation in DED and result in a better response to treatment. 0.05% CsA eyedrops are recommended as a safe and efficacious therapeutic alternative for patients with mild DED who fail to respond to AT therapy.

Abbreviations

CsA	Cyclosporine A
FML	Fluorometholone
AT	artificial tears
OSDI	Ocular Surface Disease Index
NIBUT	non-invasive tear break-up time
CFS	corneal fluorescein staining
CGC	conjunctival goblet cell
IOP	intraocular pressure
BCVA	Best corrected visual acuity
DCs	dendritic cells
DED	dry eye disease
FDA	Food and Drug Administration
IVCM	in vivo confocal microscopy
PAS	periodate Schiff
CNFD	cornea nerve fiber density
CNFL	cornea nerve fiber length
CNFW	cornea nerve fiber width
ANOVA	Repeated Measures Analysis of Variance

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Author contributions

RHW conceived and supervised the experiment. HJG, AXD and XZ performed the study and collected the data. LZ, MDC and LL analyzed the data, LZ wrote the manuscript and EEP guided the article revision.

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Data availability

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Declarations

Ethics approval and consent to participate
The research was approved by the Ethics Committee of Tianjin Medical University Eye Hospital (ethics approval number: 2021KY-17). Written informed consent was obtained from all enrolled participants.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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