

# A double-masked comparison of 0.1% tacrolimus ointment and 2% cyclosporine eye drops in the treatment of vernal keratoconjunctivitis in children

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## Summary

**Objective:** To compare the efficacy of 0.1% tacrolimus (FK-506) ophthalmic ointment with 2% cyclosporine eye drops in the treatment of vernal keratoconjunctivitis (VKC).

**Design:** Prospective double-masked randomized comparative trial.

**Participants:** Twenty-four VKC patients were enrolled into the study. Their mean age was  $9.61 \pm 2.55$  years. Twelve patients were allocated into the FK-506 group and the other twelve into the cyclosporine group. Their baseline characteristics were similar between groups.

**Methods:** After a 2 week run-in period, patients were randomized into 2 groups in a double-masked, parallel fashion. Group A received 0.1% FK-506 eye ointment twice daily with placebo eye drops four times daily for 8 weeks. Group B received 2% cyclosporine eye drops with placebo ointment for the same duration. All patients received an open-treatment with 0.1% FK-506 eye ointment for another 4 weeks. Subjective ocular symptoms and side effects were recorded by patients once daily, during the entire period. Objective ocular signs were evaluated and scored at each follow up visit.

**Main Outcome Measure:** Improvement of total subjective symptom scores per day (TSSS) within group and between groups at various time points.

**Results:** For within group comparison, there was a significant decrease in TSSS, compared to their baselines, at weeks 4 and 8, in both treatment groups. However, no statistical difference in TSSS was noted between groups at any time point. Total ocular sign scores (TOSS) in the FK-506 group decreased significantly at weeks 4 and 8 compared to baseline. Although there was a decrease of TOSS in the cyclosporine group, the difference did not reach statistical significance. Side effect scores reduced significantly in both groups at week 4 compared to their respective baselines ( $p = 0.034$  in the FK506 group and  $p = 0.003$  in the cyclosporine group). There was no significant difference in the comparison between groups on TOSS and side effect scores at any time point of the study. During the open FK-506 period, patients in cyclosporine group showed further reduction of both TSSS and TOSS. However, these changes were not statistically significant ( $p > 0.05$ ).

**Conclusion:** 0.1% FK-506 eye ointment and 2% cyclosporine were both effective in treatment of VKC. 0.1% FK-506 could become another viable therapeutic option for VKC. (*Asian Pac J Allergy Immunol 2012;30:177-84*)

**Key words:** VKC, FK-506, tacrolimus, cyclosporine, children

## Abbreviations

AKC	=	Atopic keratoconjunctivitis
FK-506	=	Tacrolimus
SPT	=	Skin prick test
TSSS	=	Total subjective symptom scores
TOSS	=	Total ocular sign scores
VKC	=	Vernal keratoconjunctivitis

## Introduction

Vernal keratoconjunctivitis (VKC) is a chronic sight-threatening inflammatory eye disease commonly observed in children and adolescents. VKC usually occurs before 10 years of age. The disease generally

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lasts 2-10 years and ordinarily resolves by puberty. Male-to-female ratio is about 2:1. The disease is observed predominantly in warm climate such as in countries surrounding the Mediterranean, in West African nations, in the Middle-East and in Japan.<sup>1</sup> Three forms of the disease are observed, i.e., limbal, tarsal and mixed VKC.<sup>2</sup> Symptoms of VKC include intense itching, tearing, mucous secretions and severe photophobia.<sup>3,4</sup> Conjunctival signs comprise hyperemia, papillary hypertrophy, giant papillae, discharge, and Horner-Trantas dots (accumulation of gelatinous inflammatory infiltrates around the limbus). A significant history of atopy is found in three-quarters of VKC patients and a family history of atopy is observed in about two-thirds. Since negative skin test is not infrequently observed among VKC cases, VKC cannot be regarded as a disease solely due to hypersensitivity type-1.<sup>5</sup> Histopathologically, VKC is characterized by conjunctival infiltrations with eosinophils, degranulated mast cells, basophils, plasma cells, lymphocytes, and macrophages. T cell culture from conjunctival scraping of VKC patients yielded mainly Th2-type clones.<sup>6</sup> Th2-derived cytokines such as IL-4, IL-5, IL-13, growth factors and enzymes are found in the conjunctiva of VKC patients.<sup>4</sup> Increased production of Th2 cytokines in VKC may contribute, in part, to tissue remodeling and papillary formation on tarsal conjunctiva.<sup>7</sup>

More than half of VKC patients developed corneal lesions such as superficial punctate keratopathy, corneal erosion, persistent corneal epithelial defects, corneal ulcers, and corneal plaque.<sup>8-9</sup> Vision can be severely impaired.<sup>10</sup> Exacerbations of VKC are often controlled by topical steroids which could produce serious side-effects such as glaucoma, cataracts and ocular infections. Steroid-induced glaucoma is not uncommon and could lead to blindness.<sup>11</sup>

Cyclosporine and tacrolimus (FK-506) inhibit activation of T cells, and also inhibit IgE-dependent histamine release from mast cells and basophils.<sup>12</sup> Both drugs act on their target cells via cyclophillin receptors. Cyclosporine eye drops were found to be effective in treating severe VKC.<sup>13</sup> However, burning with 2% cyclosporine was unacceptable to several patients and could lead to low compliance.<sup>14-17</sup> Tacrolimus (FK-506) is a macrolide antibiotic that has potent immunomodulatory properties.<sup>18</sup> It acts primarily on T-lymphocytes by inhibiting production of cytokines, particularly IL-2, IL-3, IL-5, TNF- $\alpha$  and IFN- $\gamma$ .<sup>14</sup> 0.1% tacrolimus ophthalmic ointment preparation was initially examined in our 4-week,

open-label trial among 10 recalcitrant VKC patients as an alternative treatment to cyclosporine with satisfactory results.<sup>19</sup> Adverse effect from 0.1% tacrolimus ointment in that study was only transient stinging effect which diminished shortly after the initiation of the trial. Systemic absorption of FK-506 from conjunctival surface was found to be minimal.<sup>19</sup>

In this study, we compared the efficacy of 0.1% tacrolimus ophthalmic ointment to that of 2% cyclosporine eye drops in the treatment of VKC.

## Methods

### Study Population

VKC patients presented to the pediatric allergy clinic and to the ophthalmology clinic at the Faculty of Medicine Siriraj Hospital, Mahidol University, during June 2003 to May 2005, were recruited into the study. Diagnosis of VKC was made clinically by our ophthalmologist (PK), according to commonly accepted criteria.<sup>1-4</sup> All patients had active disease on enrollment. Exclusion criteria were presence of co-existing ocular diseases such as glaucoma, uveitis, corneal disease, ocular infection, presence of systemic diseases other than co-existing allergic rhinitis, asthma and atopic dermatitis and reported hypersensitivity to FK-506 or cyclosporine. Based on results of previous studies on efficacy of treatment of VKC by cyclosporine (50% symptom reduction)<sup>13</sup> and tacrolimus (80% symptom reduction)<sup>19</sup> at 2 weeks of treatment, 12 patients were required in each study arm to provide a statistical difference of 0.05 and with the power of 80%. The study proposal was reviewed and approved by the Human Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University. Informed consent was obtained from parents and also from patients when appropriate. The trial was registered with ClinicalTrials.gov and is published on ClinicalTrials.gov public site (ClinicalTrials.gov Identifier: NCT01068054).

### Study medications

Both tacrolimus eye ointment and cyclosporine eye drops used in this study were prepared by a hospital pharmacist at Siriraj hospital under a good clinical practice guideline. Topical 0.1% FK-506 eye ointment was prepared from oral tacrolimus-capsules (Prograf<sup>TM</sup> Fujisawa, Japan) mixed with ophthalmic base containing 80% paraffin, 10% liquid paraffin, and 10% lanolin. The ointment was packed under a sterile condition into 5-gram ophthalmic tube dispensers. The ointment were used



successfully in our initial open-label trial in ten VKC patients without any side-effects.<sup>19</sup> 2% cyclosporine were prepared using cyclosporine (50 mg/ml) dissolved in olive oil under a sterile condition. Identical placebo ointment and eye drops were prepared using solvent as placebo. All preparations were individually prepared for each patient and were discarded at the end of study. All preparations were blindly coded from both patients and investigators. All drugs were accounted for during each visit by the investigators.

### Study protocol (see Figure 1)

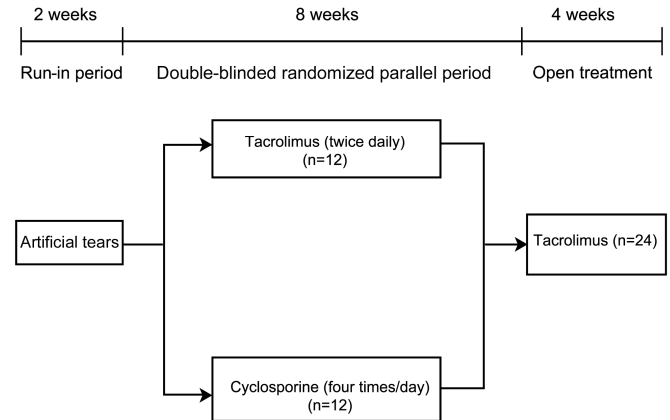
**Visit 1** (at entry): Eligible patients were enrolled into the study. Written informed consent was obtained. Patients underwent a complete general physical and ophthalmologic examination including scoring designed specifically for VKC as described in our previous study.<sup>19</sup> Ophthalmologic and oral allergic drugs, with the exception of preservative-free artificial tears and cold compress, were discontinued during the 2-week run-in period during which patients recorded eye symptom scores once daily. No oral corticosteroids were allowed. Rescue medications included only cold compress and preservative-free artificial tears.

**Visit 2** (week 0): Patients returned to the clinic after a run-in period for another complete eye examination included uncorrected visual acuities, tonometry, slit lamp bio-microscopy for grading severity of conjunctival injection, chemosis, size and amount of papillae, corneal infiltration and amount of Horner-Trantas dots. Patients were then randomized by computer-generated numbers into 2 groups.

Patients in group A received 5-gram tubes of 0.1% tacrolimus ophthalmic ointment and placebo topical eye drops. One drop of placebo eye drops was applied to each eye and followed within an hour with 0.5 cm column of active eye ointment applied onto both lower conjunctival chambers. The eye drops were applied 4 times daily whereas the eye ointment was used twice daily.

Patients in group B received 2% cyclosporine eye drops and placebo eye ointment (with the same amount and frequency of medications used as in group A).

Patients and physicians were blinded to the study drug code until the end of the study. Patients recorded and scored their symptoms before and 30 minutes immediately after administration medication once daily before bedtime. Such symptom score card enlisted common eye symptoms designed and used in previous study.<sup>19</sup>



**Figure 1.** Design of the study. Twenty-four patients were enrolled. All patients underwent a 2-week run-in period and were then randomized into 2 treatment arms (twelve patients each) for 8 weeks. At the end of the randomization period, all patients received tacrolimus ointment in an open fashion for an additional 4 weeks.

**Visit 3** (week 2), and **Visit 4** (week 4): Patients returned to the clinic for complete examination (general physical and ophthalmologic examination). Compliance was checked from diary score card and also by visually inspecting dispensers for eye ointment and eye drops.

**Visit 5** (week 8): Patients returned for complete examination, after using medication for 8 weeks. External ocular photography was taken. Patients from both groups were then prescribed 0.1% tacrolimus ophthalmic ointment for use twice daily for another open period of 4 weeks.

**Visit 6** (week 12): Patients returned for final examination.

### Clinical Scoring System

The main outcome measure was total subjective symptom scores per each day of treatment (TSSS). Secondary outcomes included changes in total objective ocular sign score (TOSS) at each visit (see below) and in side effects of medications.

Each patient recorded subjective symptom scores once daily (itching, photophobia, tearing, foreign body sensation and burning sensation) before usage of the medication. Each variable was graded as follows: 0 = absent, 1 = mild, 2 = moderate or 3 = severe.<sup>16</sup> These scores were summed and averaged to yield total score/day (TSSS). Total objective ocular signs (TOSS) were recorded at each visit. These scores comprised hyperemia of bulbar and palpebral conjunctiva, papillae, giant papillae, and corneal infiltration which were graded as suggested by

Bonini et al.<sup>20</sup> Papillae were graded according to their numbers and sizes; 0 = absent, 1 = a few papillae of less than 0.2 mm, 2 = papillae of 0.3 to 1 mm on the tarsal conjunctiva, 3 = papillae of 1 to 3 mm throughout the tarsal conjunctival area. Giant, cobblestone-like papillae (>3 mm) of the superior tarsal conjunctiva were also graded according to their numbers and sizes; 1 = few giant papillae of 3 to 4 mm, 2 = giant papillae of 3 to 4 mm throughout the tarsal conjunctival area, or a few papillae of 4 to 6 mm, 3 = giant papillae of 4 to 6 mm throughout the tarsal conjunctival area, or papillae = 6 mm. Corneal infiltrates were scored based on epithelial defects; 0 = no corneal involvement, 1 = fine superficial epithelial defects involving less than half of the cornea, 2 = diffuse fine superficial epithelial defects involving more than half of the cornea, 3 = confluent epithelial defects, mucous plaque formation or oval corneal ulcers. The eye with higher severity at baseline was selected. Objective ocular signs at each visit were summed (TOSS). Maximal values of TSSS and TOSS were 15. These scores were used for comparison within and between groups.

### Adverse reactions

Ocular discomforts from drugs such as burning, blurring, stinging, ocular pain, periorbital edema, periorbital rash and headache were evaluated within 30 minutes after administration of the medications. They were graded from 0-3 with 0 = absent; 1 = mild; 2 = moderate; or 3 = severe. As for TSSS, side effects during each week were summed and averaged (per day) and for comparison within and between groups.

All patients underwent skin prick tests for common inhalant and food allergens for Thailand using ALK-Abello diagnostic extracts (Port Washington, New York). A wheal response of 3 mm in diameter or more was considered positive.<sup>21</sup>

### Conjunctival cytology

Scraping with disposable plastic scoops (Rhinoscrape™) for cytologic examination was obtained from upper tarsal conjunctiva after anesthesia with 0.5% tetracaine hydrochloride for all patients. These examinations were performed at weeks 0, 8 and 12. Staining with a modified Wright-Giemsa stain was performed and cytologic grading as for nasal cytogram was carried out.<sup>22</sup>

### Statistical Analysis

All data were analyzed by a statistical software package (SPSS11.0, Chicago, Illinois). Comparison of TSSS, TOSS and side effect scores between and

within group at different time points (at entry, weeks 1, 4, 8 and 12) was performed by ANOVA with repeated measure analysis and with Bonferroni corrections. A *p* value of less than 0.05 was considered statistically significant. Associated allergic diseases between two groups were compared by Fisher's exact test. Comparison of cytologic gradings of eosinophils between the two groups was made by Mann-Whitney U test.

## Result

### Study population

Twenty four consecutive VKC patients presented to Siriraj Hospital during 2003-2005 were enrolled (without any drop out). Their mean age was 9.61 ± 2.55 years (median 8.90 ± 2.55 years; range 5.42 - 14.05 years, Table 1). There were 23 males and 1 female. The mean age of onset of VKC symptoms

**Table 1.** Demographic characteristics and disease severity of 24 VKC patients enrolled into the study

	Tacrolimus group	Cyclosporine group	<i>p</i> value	Total
No. of patients	12	12		24
Male	11	12		23
Female	1	0		1
Mean age ± SD (range, all in years)	10.14 ± 2.60 (5.42-14.05)	9.07 ± 2.50 (5.83-13.15)	<i>p</i> = 0.32	9.61 ± 2.55 (5.42-14.05)
Age group				
5-10 years	6	8		14
10-15 years	6	4		10
Mean age of onset (range, in years)	7.12 ± 1.80 (4.30-10.05)	5.85 ± 2.70 (1.87-9.97)	<i>p</i> = 0.44	6.49 ± 2.33 (1.87-10.05)
Duration of disease before treatment in years (range)	2.93 ± 2.14 (0.06-7.0)	3.21 ± 2.51 (1-10)	<i>p</i> = 0.38	3.07 ± 2.28 (0.06-10)
Associated atopic conditions (cases)				
Allergic rhinitis	10	9		19 (79.17%)
Asthma	3	1		4 (16.67%)
Atopic dermatitis	1	0		1 (4.17%)
Family history of VKC	0	0		0
Skin prick test				
Positive	10	11	<i>p</i> = 1.0	21
Negative	2	1		3
Type of VKC				
Limbal	1	4		5 (20.83%)
Tarsal	7	8		15 (62.50%)
Mixed	4	0		4 (16.67%)
Total subjective symptom scores – TSSS (Mean ± SD)	6.07 ± 3.95	6.05 ± 2.26	<i>p</i> = 0.97	
Total ocular sign scores -TOSS (Mean ± SD)	13.58 ± 4.48	13.55 ± 6.14	<i>p</i> = 1.00	

was  $6.49 \pm 2.33$  years (median  $6.56 \pm 2.33$  years; range 1.87 - 10.05 years) with a mean disease duration of  $3.07 \pm 2.28$  years (median  $2.56 \pm 2.28$  years; range 0.06 - 10 years). Twenty-three patients had used some form of treatment for VKC before enrollment (topical steroid - 12 patients, olopatadine - 11 patients, lodoxamine - 5 patients and 0.5% cyclosporine - 5 patients). One patient was a newly diagnosed VKC case which had not been previously treated with any drug at entry. One patient had undergone excision of giant papilla at 2 months before enrollment.

Tarsal VKC was the most common form of presentation ( $n = 15$ ) followed by limbal ( $n = 5$ ) and mixed type VKC ( $n = 4$ ). Mean symptom duration was the longest among the tarsal group ( $3.59 \pm 2.52$  years) followed by mixed type ( $2.78 \pm 1.45$  years) and limbal type ( $2.23 \pm 1.32$  years). There was no statistical difference in duration of symptoms between the three types of VKC ( $p > 0.05$ ). Mixed type had the highest TSSS and TOSS (means =  $6.57 \pm 5.10$  and  $8.00 \pm 1.22$ ) followed by tarsal type (means =  $6.10 \pm 2.62$  and  $7.13 \pm 2.69$ ) and limbal type (means =  $5.63 \pm 3.63$  and  $5.00 \pm 1.62$ ). Again, no difference between these scores was observed ( $p > 0.05$ ).

The five major complaints recorded by patients were itching (23 patients, 95.83%), eye discharge (23 patients, 95.8%), tearing (20 patients, 83.3%), photophobia (19 patients, 79.1%) and burning sensation (19 patients, 79.1%).

Associated allergic diseases were noted in 83.88% (allergic rhinitis 79 %, asthma 16% and atopic dermatitis 4%). There was no reported history of previous VKC in any family. Table 1 shows demographic characteristics of VKC patients randomized into the two treatment groups. The two groups matched well with regards to sex, mean age, age of onset of disease, duration of symptoms prior to enrollment, associated atopic conditions, positive rates of skin test and severity of disease (TSSS and TOSS) at the end of the run-in period (Table 1). By chance, four patients with mixed type VKC were randomized to the FK-506 group whereas none was placed in the cyclosporine group.

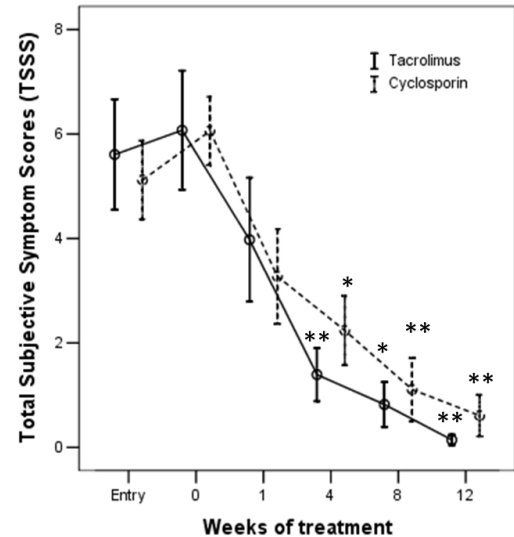
Twenty-one patients (87.5%) had positive allergy skin test. There was no difference in skin test positive rates between the FK-506 and cyclosporine groups ( $p > 0.05$ ). All patients with mixed VKC were skin tested positive whereas tarsal and limbal type yielded lower positive rates (86.7% and 80% respectively). The two most common sensitizing allergens were house-dust mites (58%) and American cockroach (33%).

### Total subjective symptom scores (TSSS - Figure 2)

Reduction of TSSS within group (compared to their baselines at the end of run-in period) became statistically significant at week 4 for FK-506 ( $p < 0.01$ ) and for cyclosporine ( $p < 0.01$ ). Such reductions were maintained in both groups throughout the 8 week period (see Figure 2). Total improvement of TSSS (as a percentage of baselines) among FK 506 was 86.49% and cyclosporine was 79.04%. Comparisons of TSSS between the two groups were shown in Figure 2.

Despite a trend for higher improvement in the FK-506, no significant difference between groups was observed ( $p > 0.05$ ). It can be noted that further reduction of TSSS among patients in cyclosporine group after switching to the open period of FK-506 treatment was observed at week 12. However, such reduction did not achieve statistical significance ( $p > 0.05$ ).

When individual symptom was examined (itching, irritation, watery eye, photophobia and burning), improvements were observed in both groups at varying degrees (data available from the authors). As with TSSS, improvements were observed after 4 weeks of treatments; no difference between treatment groups were observed ( $p > 0.05$ ).



**Figure 2.** Mean  $\pm$  SE of total subjective symptom scores (TSSS) at each time point of the two treatment groups (at entry, after run-in, and at various weeks of treatment). TSSS significantly decreased from baselines at 4<sup>th</sup> and 8<sup>th</sup> weeks in both groups, (\*,  $p < 0.05$  \*\*,  $p < 0.01$ ). No difference was observed between groups at any time point ( $p > 0.05$ ). Plots of data from the two groups, at each time point, are slightly overlapped (as in the graph function of SPSS) to allow better appreciation of data distribution.

**Total objective ocular signs (TOSS - Figure 3).**

Reduction of TOSS became statistically significant at week 4 in the FK-506 group ( $p < 0.01$ ) which was maintained through the 8<sup>th</sup> week period. Despite a reduction of TOSS within the cyclosporine group, no significant change was observed compared to baseline ( $p > 0.05$ ). TOSS in cyclosporine group continued to decrease after switching to FK506 although this was not statistically significant. No difference in TOSS between the two treatment groups was observed at any time points.

Hyperemia of palpebral conjunctiva significantly decreased in both group at week 4, whereas bulbar conjunctiva hyperemia improved only in FK506 ( $p < 0.05$ ). Improvement in giant papillae was seen in both groups although it was statistically improved only in FK-506 group at week 8 ( $p < 0.01$ ). No significant difference in papillae, giant papillae and corneal infiltration between baseline and 8 week period of treatment in the cyclosporine group was noted.

**Side effects**

Side effect scores reduced with time and became statistically significant in both groups at week 4 ( $p > 0.05$ ). Comparison of side effect scores between groups at was not significantly different any time point. Intraocular pressures, measured at all visits, were within normal limits. No serious adverse effect requiring withdrawal from the study was noted in either group. No patients developed any exacerbation of the disease during the treatment. No adjunctive therapy was required during the entire study.

**Cytologic study**

The mean number of eosinophil counts from upper conjunctiva tarsal prior to treatment group were higher in the cyclosporine group (cyclosporine group  $3.67 \pm 0.65$  and FK-506 group  $1.96 \pm 1.89$ ,  $p = 0.022$ ). These values decreased by week 8 of treatment to  $1.38 \pm 1.10$  and  $0.75 \pm 0.96$ , respectively. Such decrease, however, were not statistically significant ( $p > 0.05$ ).

**Discussion**

In this study, both topical treatment of 0.1% FK-506 ophthalmic ointment and 2% cyclosporine eye drops were effective for VKC. Despite being a double-masked study, we did not include a placebo arm since it was deemed unethical to leave patients with severe VKC untreated for such a prolonged period (14 weeks). The effectiveness of FK-506 in this study confirmed the result of our earlier open trial in 10 VKC patients.<sup>19</sup> As in our initial study, response to FK-506 could be observed as early as

after one week of treatment. Continuing improvement was observed throughout the study period including the last 4 week of open trial period (Figure 2 and 3).

Topical cyclosporine of various concentrations has been investigated for VKC treatment with varying results. In a 2-week, double-masked, placebo-controlled trial by Pucci et al,<sup>13</sup> 2% cyclosporine resulted in an approximately 40% reduction in subjective and objective scores by the end of the randomization period. 2% cyclosporine eye drop was tried in a large number of children with VKC from Rwanda, Africa.<sup>23</sup> In that study, 2% cyclosporine eye drop was shown to be as effective as topical dexamethasone during the 4-week study period. In a longer open trial by Spadavecchi et al<sup>24</sup> utilizing a lower concentration of cyclosporine (1.25% and 1%) for 4 months, a higher degree of benefit in subjective and objective was observed. Recently, the use of 1% topical cyclosporine in VKC resulted in subjective symptoms and objective signs after 2 weeks and 4 months.<sup>25</sup> Difference in efficacy of cyclosporine in various publications could be due to difference in methods for cyclosporine preparation and to difference in disease severity among patients recruited in different trials. 0.2% cyclosporine was selected for our study since this concentration was used in earlier reports.

Similar to FK506, the result from cyclosporine treatment in our study became noticeable after 2 weeks. Continuing improvement of both TSSS and TOSS in the cyclosporine could be seen throughout the 8 week randomization period (Figure 2 and 3). Moreover this group of patients (the cyclosporine group) derived further benefit after crossing over to receive FK-506 for 4 more weeks although the difference was not statistically significant ( $p > 0.05$ ). Such improvement indicated that despite the fact that both drugs (cyclosporine and FK 506) acted similarly on cyclophyllin proteins receptor, additional benefit could be obtained by interaction on different protein-drug interaction.

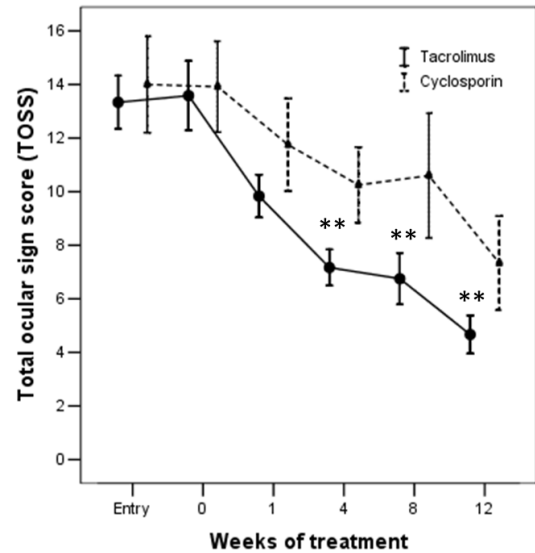
Since our earlier successful report in the use of FK506 in VKC,<sup>19</sup> several investigators have reported the use of topical tacrolimus in various ocular disorders such as anterior segment inflammatory diseases,<sup>26</sup> severe atopic blepharocon-junctivitis,<sup>27</sup> giant papillary conjunctivitis<sup>28</sup> and atopic keratoconjunctivitis (AKC).<sup>26,29</sup> Most studies used conventional tacrolimus ointment (dermatolo-gical) in their trials (0.03%). Symptoms and signs could be observed as early as 5 days to 2 weeks. A patient with giant papillary conjunctivitis entered a remission phase with complete

resolution of papilla within 1 month.<sup>28</sup> Recently, a randomized, placebo-controlled trial of tacrolimus 0.1% solution in severe allergic conjunctivitis (41 AKC and 14 VKC) was reported.<sup>29</sup> All patients responded to this novel preparation of tacrolimus. As with tacrolimus ointment, the effect from solution could clearly be seen as early as 1 week of treatment. Most interesting finding from this later report was the resolution of giant papilla with tacrolimus treatment. Such finding was seen in some of our patients in our current study as well as in our previous study, indicating that tacrolimus could potentially alter the natural course of both AKC and VKC.

Cyclosporine is notorious to cause severe eye burning upon application. In a recent 6-month study in VKC, 9.1% of patients discontinued 0.1% cyclosporine due to adverse events. The most common adverse reaction was eye irritation (4.4%).<sup>30</sup> In our study, improvement such as burning and ocular pain was observed after week 4 only with FK-506.

The limitation of our study is a small sample size. Numbers of patients included in our trial were calculated from estimated effectiveness of cyclosporine in an earlier trial<sup>13</sup> and from our open FK-506 trial.<sup>19</sup> Apparently, cyclosporine gave a better response in our study than in the study by Pucci et al.<sup>13</sup> With this reason, the result of our study could be subjected to a lower statistical power (type-II error). A difference of the efficacy between the two medications in VKC will have to be further studied in a larger trial and with standard preparation of drugs.

Since the US Food and Drug Administration put a Black-Box warning on the use of FK-506 ointment in the treatment of atopic dermatitis for its potential cause-and-effect of lymphoid malignancy, such action hindered an effort to develop FK506 ointment for ophthalmological use. Interestingly, a study of aqueous solution of tacrolimus was reported.<sup>29</sup> Such effort revives hope for patients with VKC and other related disorders. Potential side-effects of any immune modulator in the treatment of ocular disease included infections with opportunistic agents. Fungal superimposed infection was reported with the use of cyclosporine ophthalmic preparation<sup>31</sup> and herpes simplex infection with tacrolimus.<sup>26</sup> However, in our 3-year experience of FK-506 ophthalmic use (continuous and intermittent use), we did not observe any serious side-effect.<sup>32</sup> Moreover, the finding from a study by Ebihara et al,<sup>33</sup> confirmed



**Figure 3.** Mean  $\pm$  SE of total ocular sign scores (TOSS) at each time point of the study. TOSS decreased at 4<sup>th</sup> and 8<sup>th</sup> week only in the tacrolimus group (\*\*,  $p < 0.01$ ). Although further reduction of scores was more apparent in the cyclosporine group, such difference was not statistically significant ( $p > 0.05$ ). Plots of data from the two groups, at each time point, are slightly overlapped (as in the graph function of SPSS) to allow better appreciation of data distribution.

our previous finding that topical ophthalmic tacrolimus led to a minimal systemic absorption of the compound.<sup>19</sup>

In conclusion, we report that FK-506 ophthalmic ointment twice daily brought about an improvement of symptoms of VKC similar to that of cyclosporine eye drop four times daily. Objective ocular signs tended to be more improved with FK506 albeit not statistically significant. Cyclosporine eye drops was associated with a rather bothersome burning sensation and ocular pain on application whereas transient burning was observed with FK-506. Patients who received cyclosporine appeared to obtain further benefit after switching to open period with FK-506. FK-506 is a potential useful drug in the treatment of VKC.

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## Conflicts of interest

The authors have no proprietary or commercial interest in any materials used and discussed in this article.

## References

1. Collum MT. Vernal keratoconjunctivitis. *Acta Ophthalmol Scand*. 1999;228 (Suppl):14-6.
2. Neumann E, Gutmann MJ, Blumenkrantz N, Michaelson IC. A review of four hundred cases of vernal keratoconjunctivitis. *Am J Ophthalmol*. 1959;47:166-72.
3. Bielory L. Allergic and immunologic disorder of the eye. Part II: Ocular allergy. *J Allergy Clin Immunol*. 2000;106:1019-32.
4. Leonardi A. Vernal keratoconjunctivitis: pathogenesis and treatment. *Prog Retin Eye Res*. 2002;21:319-39.
5. Staphl JL, Barney NP. Ocular allergic disease. *Curr Opin Allergy Clin Immunol*. 2004;4:455-9.
6. Bonini S. Atopic keratoconjunctivitis. *Allergy*. 2004;59(Suppl. 78):71-3.
7. Cook EB. Tear cytokines in acute and chronic ocular allergic inflammation. *Curr Opin Allergy Clin Immunol*. 2004;4:441-5.
8. Cameron JA. Shield ulcers and plaques of the cornea in vernal keratoconjunctivitis. *Ophthalmology*. 1995;102:985-93.
9. Kumagai N. Epidemiology of allergic conjunctival diseases. *J Jap Ophthalmol Assoc*. 1988;69:905-9.
10. Kumagai N, Fukuda K, Fujitsu Y, Yamamoto K, Nishida T. Role of structural cells of the cornea and conjunctiva in the pathogenesis of vernal keratoconjunctivitis. *Prog Retin Eye Res*. 2006;25:165-87.
11. Tabbara KF. Ocular complications of vernal keratoconjunctivitis. *Can J Ophthalmol*. 1999;34:88-92.
12. Schreiber SL, Crabtree GR. The mechanism of action of cyclosporine A and FK 506. *Immunol Today*. 1992;13:136-42.
13. Pucci N, Novembre E, Cianferoni A, Lombardi E, Bernardini R, Caputo R, et al. Efficacy and safety of cyclosporine eye drops in vernal keratoconjunctivitis. *Ann Allergy Asthma Immunol*. 2002;89:298-303.
14. Gupta V, Sahu PK. Topical cyclosporin A in the management of vernal keratoconjunctivitis. *Eye*. 2001;15:39-41.
15. Avunduk AM, Avunduk MC, Erdol H, Kapicioglu Z, Akyol N. Cyclosporine effects on clinical findings and impression cytology specimens in severe vernal keratoconjunctivitis. *Ophthalmologica*. 2001;215:290-3.
16. Bleik JH, Tabbara KF. Topical cyclosporine in vernal keratoconjunctivitis. *Ophthalmology*. 1991;98:1679-84.
17. Secchi AG, Tognon MS, Leonardi A. Topical use of cyclosporine in the treatment of vernal keratoconjunctivitis. *Am J Ophthalmol*. 1990;110:641-5.
18. De Paulis A, Cirrillo R, Ciccarelli A, Columbo M, Marone G. FK-506, a potent novel inhibitor release of proinflammatory mediators from human Fc epsilon RI+ cells. *J Immunol*. 1991;146:2374-81.
19. Vichyanond P, Tantimongkolsuk C, Dumrongkichaiporn P, Jirapongsananuruk O, Visitsunthorn N, Kosrirukvongs P. Vernal keratoconjunctivitis; result of a novel therapy with 0.1% topical ophthalmic FK 506 ointment. *J Allergy Clin Immunol*. 2004;113:355-8.
20. Bonini S, Bonini S, Lambiase A, Marchi S, Pasqualetti P, Zuccaro O, et al. Vernal keratoconjunctivitis revisited: a case series of 195 patients with long-term follow up. *Ophthalmology*. 2000;107:1157-63.
21. The European Academy of Allergy and Clinical Immunology. Position paper: allergen standardization and skin tests. *Allergy*. 1993;48(Suppl):48-82.
22. Bonini S, Bonini S, Vecchione A, Naim DM, Allansmith MR, Balsano F. Inflammatory changes in conjunctival scrapings after allergen challenge in humans. *J Allergy Clin Immunol*. 1988;82:462-6.
23. De Smedt S, Nkurikiye J, Fonteyne Y, Tuft S, De Bacquer D, Gilbert C, Kestelyn P. Topical ciclosporin in the treatment of vernal keratoconjunctivitis in Rwanda, Central Africa: a prospective, randomised, double-masked, controlled clinical trial. *Br J Ophthalmol*. 2012;96:323-8.
24. Spadavecchia L, Fanelli P, Tesse R, Brunetti L, Cardinale F, Bellizzi M, et al. Efficacy of 1.25% and 1% topical cyclosporine in the treatment of severe vernal keratoconjunctivitis in childhood. *Pediatr Allergy Immunol*. 2006;17:527-32.
25. Tesse R, Spadavecchia L, Fanelli P, Rizzo G, Procoli U, Brunetti L, et al. Treatment of severe vernal keratoconjunctivitis with 1% topical cyclosporine in an Italian cohort of 197 children. *Pediatr Allergy Immunol*. 2010;21:330-5.
26. Joseph MA, Kaufman HE, Insler M. Topical tacrolimus ointment for treatment of refractory anterior segment inflammatory disorders. *Cornea*. 2005;24:417-20.
27. Virtanen HM, Reitamo S, Kari M, Kari O. Effect of 0.03% tacrolimus ointment on conjunctival cytology in patients with severe atopic blepharoconjunctivitis: a retrospective study. *Acta Ophthalmol Scand*. 2006;84:693-5.
28. Kymionis GD, Goldman D, Ide T, Yoo SH. Tacrolimus ointment 0.03% in the eye for treatment of giant papillary conjunctivitis. *Cornea*. 2008;27:228-9.
29. Ohashi Y, Ebihara N, Fujishima H, Fukushima A, Kumagai N, Nakagawa Y, et al. A randomized, placebo-controlled clinical trial of tacrolimus ophthalmic suspension 0.1% in severe allergic conjunctivitis. *J Ocul Pharmacol Ther*. 2010;26:165-74.
30. Ebihara N, Ohashi Y, Uchio E, Okamoto S, Kumagai N, Shoji J, et al. A large prospective observational study of novel cyclosporine 0.1% aqueous ophthalmic solution in the treatment of severe allergic conjunctivitis. *J Ocul Pharmacol Ther*. 2009;25:365-72.
31. Gupta A, Sharma AMohan K, Gupta A. Mycotic keratitis in non-steroid exposed vernal keratoconjunctivitis. *Acta Ophthalmol Scand*. 1999;77:229-33.
32. Pacharn P, Visitsunthorn N, Jirapongsananuruk O, Vichyanond P. Vernal Keratoconjunctivitis (VKC) treated with 0.1% FK-506 ophthalmic ointment: result of three years follow-up. *J Allergy Clin Immunol* 2007;119:S153.
33. Ebihara N, Ohashi Y, Fujishima H, Fukushima A, Nakagawa Y, Namba K, et al. Blood level of tacrolimus in patients with severe allergic conjunctivitis treated by 0.1% tacrolimus ophthalmic suspension. *Allergol Int*. 2012;61:275-82.