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INFLAMMATORY DISORDERS

Topical 0.03 % tacrolimus for subepithelial infiltrates secondary to adenoviral keratoconjunctivitis

Eliya Levinger · Omer Trivizki · Yonathan Shachar · Samuel Levinger · David Verssano

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Abstract
Objective To determine the safety and efficacy of topical 0.03 % tacrolimus ointment treatment for subepithelial corneal infiltrates (SEIs).
Methods This prospective non-controlled interventional case series included patients with SEIs who had been previously treated with topical corticosteroids with either no improvement or the medication being withdrawn due to associated intraocular pressure (IOP) elevation. The patients were treated with 0.03 % tacrolimus ointment twice daily for 22 weeks (including a 1-month washout). The objective data were best-corrected Snellen visual acuity (BCVA), IOP, and full ocular examination results, including SEI severity and the Schirmer test. The subjective data were the patients' responses to a questionnaire at all follow-up visits.
Results The patients consisted of five males (45 %) and six females (55 %) (mean age 50±11 years) who were followed up for an average of 22 weeks. The mean BCVA (logarithm of the minimum angle of resolution [logMAR]) before and after treatment was 0.34±0.09 and 0.08±0.04 respectively (p= 0.042). All the patients evidenced significant objective clinical improvement, and none had a severe degree of SEI at the end of the treatment. The patients reported considerable reduction in the severity of their symptoms (foreign body sensation, glare, etc.). Three patients were excluded due to side-effects (one had severe dizziness and discomfort), and their data were excluded from the study.
Conclusion Topical tacrolimus 0.03 % is a safe and effective alternative treatment in patients with SEIs who do not respond to other treatment modalities or have untoward side-effects from topical steroids.

Keywords Adenoviral keratoconjunctivitis · Subepithelial corneal infiltrates · Tacrolimus · Topical

Introduction

Epidemic keratoconjunctivitis (EKC) is most commonly caused by adenoviral serotypes 8, 19, and 37, and represents the most common of the external ocular viral infections [1, 2]. Keratitis that appears approximately 10 days after the onset of the follicular conjunctivitis may present with the formation of subepithelial corneal infiltrates (SEIs), which are usually bilateral and often asymmetrical. The SEIs have the potential to cause serious ocular morbidity in the form of reduced vision, photophobia, glare, halos, and foreign body sensation, and these problems can persist for months or even years after the initial infection [3, 4]. Histopathologic investigation of SEIs reveals lymphocytes, histiocytes, and fibroblasts that are accompanied by a disruption of the collagen fibers of Bowman's layer [4], The hypothesis of a persisting viral replication in subepithelial keratocytes, which triggers an immunologic host reaction, is supported by the clinical observation that opacities usually resolve with topical steroid treatment but recur when steroids are discontinued [3].

The role of topical anti-inflammatory agents to control the SEI patient's symptoms remains an important clinical goal in patient management. However, the use of topical steroids in this setting is controversial because of the complications of

Drs. Levinger and Trivizki contributed equally to this work

E. Levinger · O. Trivizki (✉) · Y. Shachar · D. Verssano
Department of Ophthalmology, Tel-Aviv Medical Center, Tel-Aviv, Israel, affiliated to the Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel
e-mail: omertr@gmail.com

E. Levinger · O. Trivizki · S. Levinger
Enaim Medical Center, Tel-Aviv, Israel

cataract formation, glaucoma, and superinfection associated with their long-term use [5–7]. In addition, the use of topical steroids may lead to steroid dependence in some patients [5–7].

Tacrolimus, also known as FK506, is a macrolide derived from the soil fungus *Streptomyces tsukubaensis*. Its mechanism of action is similar to that of cyclosporine, but it is described as being between 10 to 100 times more potent, despite differing chemical structure [8]. Both tacrolimus and cyclosporine inhibit B- and T-cell activity by decreasing the transcription of interleukin-2 and lymphokines. Systemic tacrolimus has been used successfully to prevent allograft rejection in liver, kidney, lung, and heart transplantation [8]. In ophthalmology, the systemic use of tacrolimus is already well-established in the treatment of immune-mediated diseases, uveitis, dry eyes related to graft-versus-host disease, corneal transplants, and ocular pemphigoid [9].

Topical tacrolimus has been successfully used "off label" as an ointment for treating ocular allergies, especially atopic blepharokeratoconjunctivitis, for high-risk penetrating keratoplasty, and for dry-eye syndrome [10]. The aim of this pilot study was to investigate both the subjective and objective efficacy of topical 0.03 % tacrolimus in patients with SEIs who had been treated with topical corticosteroids for a long period with no improvement or the medications being discontinued due to associated intraocular pressure (IOP) elevation.

Methods

Patient selection

This prospective non-controlled interventional case series study adhered to the tenets of the Declaration of Helsinki, and was approved by the Institutional Review Board/Ethics Committee of the Tel-Aviv Medical Center under protocol number 0359–09 (June 2012). Informed consent was obtained from all study participants after the nature of the study had been explained to them in detail. Our original study cohort had included 14 patients (14 eyes) who were clinically diagnosed as having unilateral SEI due to adenokeratoconjunctivitis and were treated with tacrolimus 0.03 % ointment (Protopic®; Fujisawa Healthcare Inc., Teva, Deerfield, IL, USA) twice daily for SEI secondary to adenoviral keratoconjunctivitis. Patients whom we suspected of having other possible reasons for SEIs (e.g., allergic conjunctivitis, herpetic conjunctivitis, bacterial conjunctivitis) were excluded from this study. All the patients had previously been treated with topical steroids for at least 13 months (dexamethasone sodium phosphate 0.1 %, Dr. Fischer, Brussels, Belgium, TID protocol). That treatment was discontinued either because of insufficient improvement in symptoms or because of IOP elevation in response to steroids. None of the patients had been treated with any other anti-inflammatory drugs before they started the treatment protocol that included cyclosporine drops. None of the study participants was willing to use medications to control IOP.

Methods

The treatment protocol included a 1-month period of washout after 6 weeks of tacrolimus 0.03 % ointment treatment, evaluation of the SEI status, and continuation of treatment for another 12 weeks. The overall course of treatment was 22 weeks. We used a recognized ointment (Protopic®) that is 100 times more potent than cyclosporine. Follow-up evaluations were carried out at 3, 6, 10, and 22 weeks after the initiation of treatment. The data recorded for each patient were as follows: best-corrected visual acuity (BCVA), IOP, functional acuity contrast sensitivity, and complete fundus examination. The objective parameters were evaluated by a clinical score for conjunctival injection (0=none, 1=mild, 2=severe), conjunctival chemosis (0=none, 1=mild, 2=severe), punctate epithelial keratitis (0=none, 1=mild, 2=severe), corneal subepithelial infiltrates (0=none, 1=few ≤10, 2=many>10), and Schirmer's test with topical anesthesia (0 = >15 mm, 1=5–15 mm, 2= <5 mm). All the study patients were examined by the same physician. For subjective evaluation of the treatment, the patients were asked to complete a non-validated questionnaire (consisting of seven items) before the initiation of the treatment and on every follow-up visit, grading their symptoms and overall satisfaction with treatment on a scale of 1 to 10 (Table 1).

Statistical methods

The patients' decimal BCVAs were converted to a logMAR scale for analysis. The data were analyzed using SPSS (Version 22 for Mac, IBM Inc.) employing the Wilcoxon signed-rank test and one-way analysis of variance as appropriate.

Table 1 Patients' baseline characteristics

Variable	*N* (%)
Total number	11 (100 %)
Age, years	
Mean ± SD	50±11
Range	29–62
Gender	
Male	5 (45 %)
Female	6 (55 %)
Eye	
Right	5 (45 %)
Left	6 (55 %)

Table 2 Ocular examination before and after treatment (11 eyes)

Variable	Before treatment	Washout[a]	After treatment	*P* value
BCVA (logMAR, mean ± SD)	0.34±0.09	0.07±0.19	0.08±0.04	0.042
SEQ (D), mean ± SD	−1.55±2.73	−1.34±3.39	−1.33±2.63	0.08
IOP, mm Hg (mean ± SD)	15±8.3	14±1.01	14±3.4	0.87
Conjunctiva hyperemia, *n*	4 (36 %)	2 (22 %)	0	
Conjunctiva chemosis, *n*	2 (18 %)	2 (22 %)	0	
SPK, *n*	8 (72 %)	4 (36 %)	0	
SEI>10, *n*	3 (27 %)	2 (22 %)	0	
Schirmer test<5 mm, *n*	2 (22 %)	2 (22 %)	0	

BCVA best-corrected visual acuity, *SEQ* spherical equivalent, *D* diopters, *IOP* intraocular pressure, *SPK* superficial punctate keratitis, *SEI* subepithelial infiltrates

[a] None of the washout period parameters was significantly different from the baseline values

Results

Demographics and objective findings

Table 1 lists the patients' age, gender, and affected eye contrast sensitivity. Table 2 provides their visual acuity and ocular examination findings before and after treatment. There was a significantly improved decrease in LogMAR BCVA, from a mean value of 0.34±0.09 to 0.08±0.04, ~2 Snellen lines, $z=2.03$, $p=0.042$). All other objective physical findings, including conjunctival hyperemia, conjunctival chemosis, punctate keratitis, and Schirmer test results showed a considerable reduction in SEIs. Figure 1 depicts slit-lamp photographs of one patient at the initiation and at the termination 22 weeks later of tacrolimus 0.03 % ointment treatment.

Figure 2 shows contrast sensitivity during treatment. The patients demonstrated a significant ($p<0.05$) improvement in contrast sensitivity of the high spatial frequencies (greater than 18 cycles per degree) only when the final follow-up examination was compared to the baseline values. There were no other significant improvements during the follow-up period.

Subjective findings

Figure 3 displays the data retrieved from the pre- and post-treatment questionnaires: the scores yielded a trend towards improvement in all the variables that were queried, including severity of symptoms, foreign body sensation, glare sensation, improvement in vision, and overall satisfaction. Interestingly, the patients' scores deteriorated at the follow-up visit after washout and improved after treatment was resumed, although there were no comparable changes in the objective physical examination findings.

Side-effects

Tacrolimus ointment and drops can cause several side-effects, such as warmness in the eye, eye irritation, pain, conjunctival hyperemia, and foreign body sensation [10]. During the first 2 weeks into the study, five out of the 11 participants reported some eye irritation that eventually resolved spontaneously. There were no side-effects that required withdrawal from the study. However, three of the 14 patients who had been diagnosed with SEI secondary to adenoviral keratoconjunctivitis and had originally been recruited to the study were subsequently dropped from the study due to various side-effects. One of them was admitted to the neurology ward for severe dizziness: a complete work-up failed to find any neurological etiology. The symptoms were relieved after discontinuation of the ointment application, and the patient was asked to re-enter the study. Symptoms of dizziness recurred 48 hours after the second initiation of treatment, whereupon the tacrolimus treatment was stopped and the patient's data were excluded from

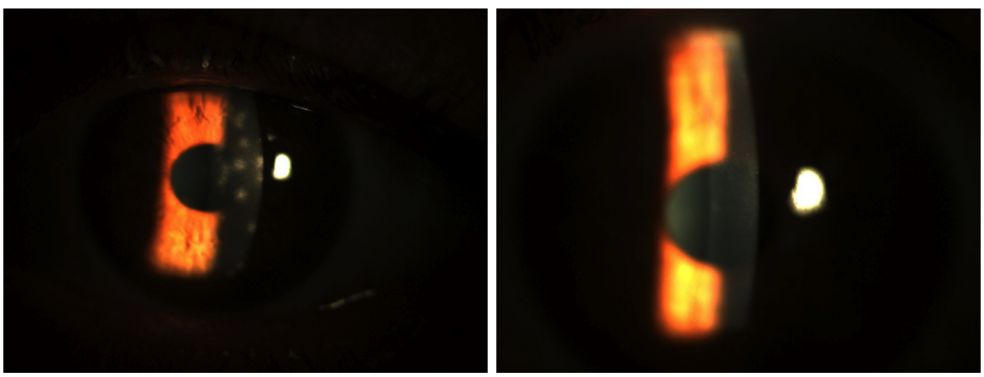

Fig. 1 Slit-lamp photographs of one patient at the initiation and at the termination 22 weeks later of tacrolimus 0.03 % ointment treatment

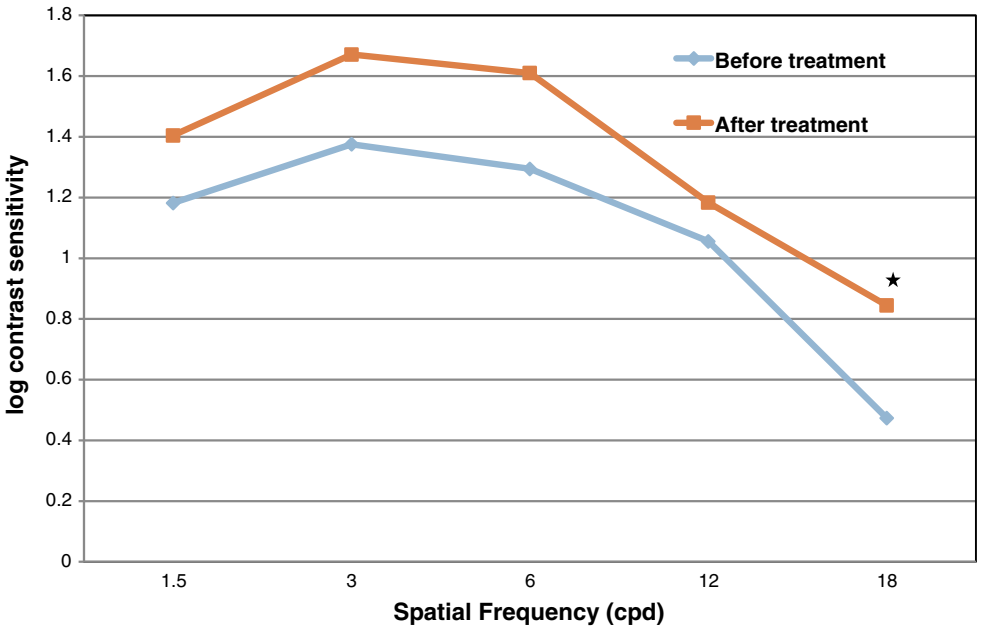

Fig. 2 Contrast sensitivity during treatment

analysis. The two other patients declined to continue treatment due to warmness in the eye, eye irritation, and a sticky sensation in the eye.

Discussion

Subepithelial infiltrates caused by adenoviral infection are a common chronic ocular condition that typically presents with severe symptomatology and is considered to be a complication of adenoviral keratoconjunctivits [11]. Currently, in the absence of an effective antiviral for adenovirus in the acute phase, therapy is often supportive and includes conservative measures, such as antihistamine and non-steroidal anti-inflammatory agents (NSAID) [2, 12]. Until recently, community ophthalmologists routinely prescribed topical corticosteroid eye drops for symptomatic relief to their patients who had acute infection [13]. It is now known that topical steroids in the acute phase increases the replication rate and disease duration; therefore, its use is usually restricted to patients whose condition is complicated by SEI or pseudomembranes [14]. In an effort to avoid the appearance of SEIs, 0.5 % topical cyclosporine A in artificial tears has been used in rabbit studies and succeeded in reducing their incidence [15]. A subsequent randomized clinical trial on humans demonstrated that the use of cyclosporine A in combination with cidofovir (an antiviral drug) did not significantly diminish the incidence of SEI compared with cidofovir alone [1].

Tacrolimus (previously known as FK-506) is an immunosuppressive drug that is mainly used after allogeneic organ transplant [16]. Its topical ointment preparation, Protopic®, was approved for the treatment of moderate-to-severe atopic dermatitis by the US Food and Drug Administration in December, 2000. In addition to its action against T-cell proliferation, in-vitro tacrolimus demonstrated a direct inhibitory effect on mast-cell degranulation. It also seems to inhibit the production of the proinflammatory mediator, interleukin 8 (IL-8), and the IL-8 receptor, as well as to decrease the binding

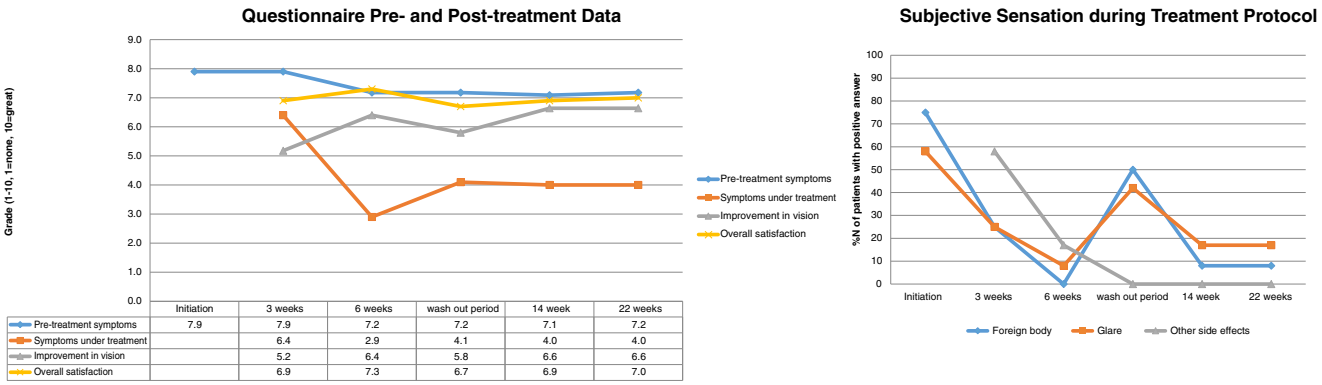

Fig. 3 Data from pre- and pos-treatment questionnaires

of IL-8 to its receptor on keratinocytes [17]. The results of in-vitro studies also suggested that tacrolimus enhances the action of the tumor suppressor gene, p53 [17].

Off-label use of tacrolimus has been described in the literature for the treatment of various conditions, such as pyoderma gangrenosum [16] and resistant chronic external otitis [18], and several studies have been published on its use for ocular indications. Kymionis et al. recently reported two cases of refractory phlyctenular keratoconjunctivitis treated with 0.03 % tacrolimus ointment: both patients showed improvement in symptoms and signs within 1 week of treatment initiation, and a complete resolution after 3 weeks of treatment [19]. Tacrolimus has also been used in penetrating keratoplasty among high-risk patients who showed signs of acute rejection: the median treatment time was 22.6 months (range 13–32), and there were no further episodes of graft rejection during the course of treatment [16]. Attas-Fox et al. reported an open-label study on 20 patients with intractable allergic conjunctivitis who were treated with tacrolimus 0.03 % for 8 weeks [20] Those authors observed significant improvement in all parameters that were checked ("conjunctivitis score").

One of our patients experienced severe dizziness that was attributed to the use of tacrolimus 0.03 % ointment. This is listed as a rare side-effect in the manufacturer's drug insert. Systemic absorption of the agent is reportedly extremely low, and the treatment is considered as being safe [21]. Ebihara et al. reported low blood drug levels in the use of tacrolimus ophthalmic suspension in allergic conjunctivitis patients [22, 23]. In 2003, an FDA advisory committee recommended that the manufacturer, Fujisawa, revise the product insert to inform patients of cancer risks from this product. In 2005, an FDA black box warning of cancer risks was required for topical tacrolimus ointment (http://www.fda.gov). The relevance of this black box warning in ocular use is not known. Indeed, tacrolimus has been used successfully in Japan for the past few years to treat uveitis without any reported adverse effects, and it has recently been accepted worldwide for treating uveitis [9].

Our adenoviral keratitis patients experienced significant improvement in their objective eye examinations with the use of tacrolimus ointment, and overall patient satisfaction and subjective evaluation of vision improvement with tacrolimus were high. There was a significant improvement in our cohort's mean logMAR BCVA (~2 Snellen lines, $p=0.042$) at the end of the 22-week course of treatment. None of our patients reported a foreign body sensation, glare or other ocular side-effects associated with topical tacrolimus treatment. We expected to see both subjective and objective deterioration during the scheduled washout period: the patients' scores did deteriorate at the follow-up visit, but there was no significant reduction in BCVA or in any of the other objective ocular findings.

Our study has several limitations. One is the small size of our study population, which was due to the fact that tacrolimus ointment is an off-label drug for ocular use and to the difficulty in finding patients willing to participate in our study. Another limitation is our use of a non-validated questionnaire to assess patient satisfaction with the treatment.

In conclusion, we found that topical tacrolimus 0.03 % was safe and effective in treating a small number of patients with SEIs. Further prospective blinded randomized studies with larger patient populations are needed to evaluate the effects of topical tacrolimus in SEIs caused by adenoviral keratoconjunctivitis and other ocular conditions.

Conflict of interest The authors did not receive any financial support from any public or private sources. The authors have no financial or proprietary interest in a product, method, or material described herein.

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