Original Article

Comparison of the safety and efficacy of topical Tacrolimus (0.03%) versus dexamethasone (0.05%) for subepithelial infiltrates after adenoviral conjunctivitis

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Purpose: To compare the safety and efficacy of tacrolimus 0.03% ointment with dexamethasone 0.05% ointment for subepithelial infiltrates (SEIs) following adenoviral keratoconjunctivitis (AK). **Methods:** A randomized, double blind trial was done. Eligibility criteria was corrected distance visual acuity of 6/9 Snellen or worse for at least 4 weeks with corneal SEIs following AK. The grading of SEIs was done on a scale of 0 to 3; 0, no infiltrates, 1 mild infiltration, 2 moderate infiltration and 3, severe infiltration. Consecutive patients with SEIs following AK were randomized to receive either topical tacrolimus 0.03% or dexamethasone 0.05% ointment twice daily for 6 months. Treatment was successful if there was reduction of SEIs and improvement in vision. **Results:** A total of 45 patients each were assigned to the Tacro and Dexa groups, respectively. Baseline characteristics of patients did not differ significantly ($P > 0.001$). There was a significant change in symptoms, vision and SEIs in both the groups. However, the magnitude was greater in tacro group. Treatment was successful in 37 (92.5%) patients in Tacro and 34 (85%) patients in dexa group. In dexa group, after a period of 1.24 ± 0.24 months, 7 (15.6%) patients developed a significant rise in intraocular pressure (IOP). Three (7.5%) eyes in tacro and 6 (15%) eyes in dexa group had recurrence of SEIs after cessation of therapy. **Conclusion:** Tacrolimus 0.03% is an effective alternative to dexamethasone 0.05% with low recurrence rate, no significant rise in IOP but may cause burning and foreign body sensation in some patients.

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Adenoviral keratoconjunctivitis (AK) usually occurs as epidemics; serotypes 8 and 19 cause most outbreaks. Infection is usually transmitted through fomites or contaminated body fluids. The virus has been demonstrated in tears for up to 3 weeks after infection.[1] The cornea is usually involved 2 or 3 days after the onset of symptoms and most common presentation is multifocal subepithelial infiltrates (SEIs), which are considered pathognomonic of adenoviral infection.[2] In subcontinent countries, SEIs may be observed in about 50% of AK cases. These focal lesions may represent a cellular immune reaction against viral antigens deposited in the corneal stroma under the Bowman membrane.[3] Histopathologically, SEIs show disruption of collagen in the Bowman layer along with infiltration of lymphocytes, histiocytes, and fibroblasts; these are usually bilateral and often asymmetric and have the potential to cause significant ocular morbidity, reduced vision, photophobia, glare, halos, and foreign body sensation and can persist for months or years after the initial infection.[4,5]

Although, AK is a self-limiting disease, most affected individuals seek treatment due to diminution of vision from persistent SEIs, pseudomembranes and iridocyclitis.[6,7] Various modalities have been tried as treatment options for AK including palliative therapy, such as cool compresses, artificial tears, and topical steroids; it is believed that steroids, by suppressing conjunctival and corneal inflammation, provide symptomatic relief, but they do not shorten the course of the disease. The use of long-term topical steroids may be associated with side effects such as cataract and glaucoma, and topical administration of corticosteroids may also cause prolonged viral seeding.[8]

Tacrolimus exerts potent immunosuppressive and anti-inflammatory effects through the inhibition of T-cell activation; it suppresses the immune system and the inflammation by inhibiting an enzyme (calcineurin) crucial for the multiplication of T-cells.[9]

Topical application of tacrolimus (0.03%) has been found to be effective in treating giant papillary conjunctivitis and vernal keratoconjunctivitis.[10,11] However, it's safety profile in ophthalmic applications need to be evaluated further. The present study evaluated the safety and efficacy of tacrolimus (0.03%) and dexamethasone phosphate (0.05%) ointment in patients with SEIs following AK.

Methods

Trial population

Across 3 referral eye centers in the subcontinent, 126 patients were diagnosed with AK from January 2015 through September

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2017. Out of these, 110 (87.3%) met none of the exclusion criteria and were asked to attend an eligibility-confirmation visit approximately 5 days later. During this period, these patients were provided with a run-in antibiotic ointment. Of the 102 patients who returned for the eligibility confirmation visit, 96 (94.1%) were eligible for inclusion in the trial. The institutional review boards and the local ethics committee approved the trial. Written informed consent was obtained from all patients willing to participate in the study based on the tenets of the declaration of Helsinki.

Eligibility criteria

Eligibility criteria were an age of 18 years or older and diminution of vision (corrected distance visual acuity of 6/9 Snellen or worse) for at least 4 weeks with SEIs following AK. SEIs were graded on a scale of 0 to 3 by an independent investigator who was not a study surgeon on slit-lamp biomicroscopic examination (depending on depth of stromal infiltration, Fig. 1); 0, no infiltrates, 1 mild infiltration, 2 moderate infiltration and 3, severe infiltration. Fig. 2a depicts moderate stromal in infiltration following AK. Patient's symptoms were evaluated by a non-validated questionnaire (Dry Eye Scoring System, DESS). The minimum score for inclusion was 1 (i.e. any symptomatic patient). A score of 0-3 was assigned to common symptoms like blurring of vision, itching or burning, sandy or gritty sensation, and redness, respectively (DESS©). When symptoms are absent, the score was (0), sometimes present (1), frequently present (2), and always present (3). A score of 0-6 was mild, 6.1- 12 moderates, and 12.1-18 severely symptomatic patient.[12]

Exclusion criteria

Patients who received any topical or systemic medications, had ocular disease like uveitis, glaucoma, or active keratitis, used corticosteroid eye drops; and those who had any ocular operations were excluded from the study.

Randomization, masking, and sample size calculation

To calculate the sample size and to compare the mean difference in SEI scores between the 2 groups, a pilot study was first done on 10 subjects. The mean decrease in SEI score in the tacrolimus group was 1.8 and, in the dexamethasone, group was 1.5, respectively. The common SD was 0.4. Assuming 1:1 randomization, 90% power (alpha = 0.05), and a precision error of 5% to detect difference of 20% or more in SEI score between 2 groups, the estimated sample size in each group was calculated to be 38 (https://www.stat.ubc.ca/~rollin/stats/ssize/n1.html).

Trial groups

Consecutive patients with SEI were randomly allocated to 1 of the 2 groups by a parallel assignment (1:1). The allocation codes were generated by a web-based module and was stratified according to clinical center with a permuted block method with randomly chosen block sizes. The generated codes were sealed in green envelopes and were opened by health care personnel not involved in patient care. The TACRO group received tacrolimus 0.03% ointment [TALIMUS-LS] twice daily and DEXA group received dexamethasone 0.05% ointment [ORBIDEX] twice daily for 6 months. The subjects were masked to the contents. The 2 types of ointments were like each other in appearance. The subjects were instructed to return the empty tubes on monthly visit, wherein 1 pack of ointment was provided to them. The regimen was reduced in frequency or suspended when the patient reported any symptoms or when a contraindication to treatment to any of active ointments developed. With resolution of symptoms or contraindications, the patient could restart or resume the regimen.

Outcome measures

The primary outcome measure was mean change in baseline in the SEI score. Changes in visual acuity and intraocular pressure (safety outcomes), and the incidence of adverse events were secondary outcome measures. Coordinators asked patients about adverse events during each visit (at 1, 3, and 6 months). Grading of SEIs and measurements of intraocular pressure (IOP) was performed by an independent investigator (AC) who was not a study surgeon; intraocular pressure was measured with non-contact tonometer (CT-60, Topcon Corporation, Japan) on each monthly visit (1,2,3,4,5 & 6 months). All patients, clinical staff, and laboratory personnel were unaware of the trial-group assignments.

Statistics

Statistical analysis was performed on an intent-to-treat basis using IBM, SPSS Statistics version 25 (IBM Inc.). One eye of each patient was selected at random for examination and subsequent evaluation. Independent t tests were performed to ensure group similarities at baseline; the assumptions of performing t tests were met. Chi-square tests were used for proportions. A one-way repeated-measures analysis of variance (ANOVA) was conducted to determine whether there were significant differences in mean test values over the course of 6 months of treatment. The values used for assessing change were the means of values obtained during the 3-month and 6-month visits; if a value from only one of these visits was available, that value was used. Comparisons of the mean change in continuous measures between trial groups and associated 95% confidence intervals were based on linear regression with a robust variance estimator. Differences between trial groups in the cumulative proportion of patients with an adverse event were evaluated with the log-rank test; Fisher's exact test was used when the number of patients in a group with a given adverse event was 3 or fewer.

Results

Patients and adherence

A total of 45 patients were assigned to the Tacro group and 45 to the Dexa group. However, after excluding 5 patients in each group (adverse effects), 40 cases in each group were analyzed for results statistically. There were no significant imbalances between trial groups in baseline characteristics [Table 1]. The mean duration of follow up was slightly longer in Dexa group (9.4 ± 1.5 vs 9.9 ± 1.4, paired *t*-test, $P = 0.010$). There was a significant improvement (paired *t*-test, $P < 0.001$) in symptoms, visual acuity (converted to Log MAR units for comparison) and SEI scores in Tacro group [Table 2] and Dexa group [Table 3], respectively. The mean symptom score decreased significantly by approximately 3 points in each group, during follow up, with greater improvement by 0.4 points in tacro group (95% CI, 2.7 to 3.8, $P = 0.001$). The mean Log MAR CDVA decreased by 0.22 points in each group with a greater change by 0.18 points in tacro group (95% CI, 0.22 to 0.27, $P < 0.001$). The mean SEI score decreased by 3 points in Tacro group and 1.5 points in dexa group, with a greater improvement by 1.5 points in tacro

group (95% CI, 1.96-2.14, $P = 0.001$). In tacro group, 34 (85%) patients had complete resolution of symptoms at final follow up examination [Fig. 2b] as compared to 30 (75%) in dexa group, respectively. Fig. 3 depicts the change in symptom score, vision, SEI Score and intraocular pressure in the two groups.

Adverse effects

In tacro group, 8 (17.8%) patients could not tolerate the medication due to burning, redness and foreign body sensation in eyes. The dose of medication was reduced to once daily at bedtime. However, 5 (11.1%) patients declined to continue the medication beyond one month due to persistent ocular symptoms and were excluded from the study; the treatment was successful in the remaining three patients who could continue with twice daily medication at three months.

In dexa group, after a period of 1.24 ± 0.24 months, 7 (15.6%) patients developed rise in intraocular pressure (IOP) after topical medication [Fig. 3]. These patients were referred to

Table 1: Baseline characteristics of patients

Variable	Tacro Group	Dexa Group
Age (mean±SD, range), years	26±4.1, (21-36)	25.4±3.7, (20-34)
Sex (*n*, %)		
Male	18 (42.9)	23 (54.8)
Female	22 (52.4)	17 (40.5)
Follow up (months)	9.5±1.48	9.9±1.4
IOP (mm of Hg)	15.7±1.8	15.6±2
Symptom Score	3.6±2	3.5±1.9
SEI Score	2.2±0.27	2.1±0.3

*Plus-minus values are means±SD. Baseline values were the means of values obtained during them screening and eligibility-confirmation visits. Symptom score range from 0-18, with a score of 0 indicating no ocular discomfort and higher scores indicating greater symptom severity. Subepithelial infiltrate score (SEI) range from 0-3, with higher scores indicating increased infiltration

Table 2: Mean test values in tacro group

Paired variable (baseline-final)	Mean difference	95% CI	Sig. (2-tailed)
Symptom Score	3.2±1.8	2.7-3.8	0.000
Log MAR vision	0.25±0.07	0.22-0.27	0.000
SEI Score	3±0.28	1.96-3.1	0.000
IOP (mm Hg)	0.07±2.4	-0.7-0.86	0.848

*Logarithm of the Minimum Angle of Resolution (Log MAR), Intraocular Pressure (IOP), Subepithelial Infiltrate Score (SEI)

Table 3: Mean test values in dexa group

Paired variable (baseline-final)	Mean difference	95% CI	Sig. (2-tailed)
Symptom Score	3±1.96	2.4-3.6	0.000
Log MAR vision	0.19±0.11	0.15-0.22	0.000
SEI Score	1.8±0.31	1.69-1.89	0.000
IOP (mm Hg)	-1.17±2.17	-1.86-0.48	0.001

Intraocular Pressure (IOP), Subepithelial Infiltrate Score (SEI)

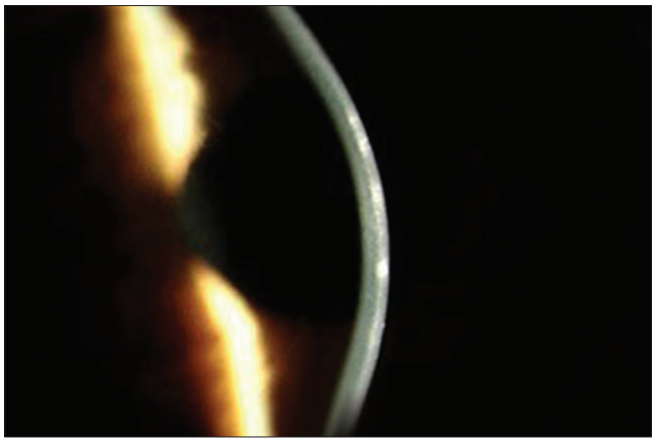

Figure 1: Moderate stromal infiltration (Grade 2) in a patient following adenoviral conjunctivitis

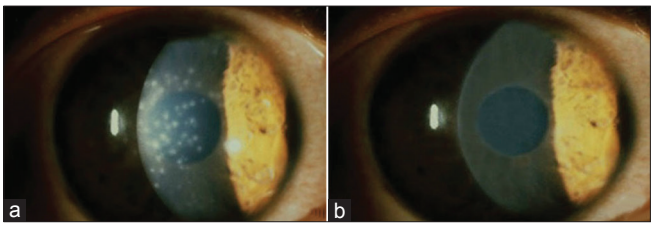

Figure 2: Corneal sub-epithelial infiltrates involving the visual axis (a)before and (b) following intervention

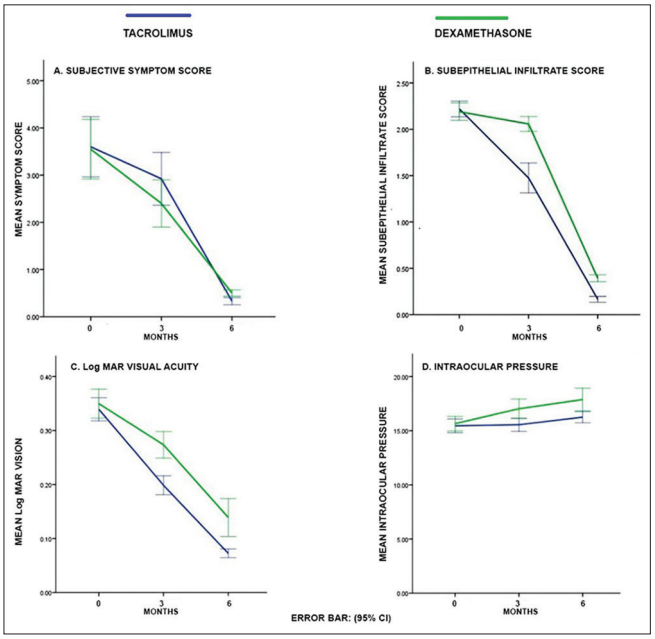

Figure 3: Subepithelial infiltrates (SEIs) were graded on a scale of 0 to 3 with higher scores indicating greater involvement. Subjective symptoms were graded on a score of 0-18 with higher scores indication sever symptoms.[12] In each trial group, there was a significant change between baseline and 6 months (with time as a continuous variable) in symptoms, vision and SEI score ($P < 0.001$ for change for each measure in each group). In dexa group, there was a significant increase in intraocular pressure

glaucoma clinic for control of IOP. Out of these 5 (11.1%) patients had sustained rise in IOP and were advised to discontinue further treatment with dexamethasone. These patients were subsequently put on treatment with tacrolimus and tolerated the therapy well but were excluded from the study. In tacro group, no statistically significant changes in IO*P* values were observed when comparing the before-treatment means (mean, 15.8 ± 1.9; range, 12-19 mm Hg) to the measurement at the last follow-up (mean, 15.7 ± 1.8 6; range, 11--17 mm Hg) (paired *t*-test, $P = 0.848$).

Recurrence

Treatment was considered successful in 37 (92.5%) patients in Tacro group and 33 (82.5%) patients in dexa group with adequate control of SEIs during follow up [Fig. 2b]. However, 3 (7.5%) eyes in tacro group and 7 (17.5%) eyes in dexa group had recurrence of SEIs after cessation of therapy. In recurrent cases in tacro group, tacrolimus (0.03%) was reintroduced in combination with topical steroids, which were tapered over a period of one month. Tacrolimus ointment was maintained for 6 months in 2 eyes and for one year in one eye without recurrence of SEIs after cessation of drug. In dexa group, dexamethasone 0.05% ointment was reintroduced, and 2 eyes were maintained for 6 months without recurrence of infiltrates. However, tapering was unsuccessful in 5 eyes on two attempts and were shifted to treatment with tacrolimus recently.

Discussion

Corneal SEIs following AK are bothersome for patients as they may persist for months or years after initial infection, causing significant ocular morbidity.[13] Chronicity of the disease is often compounded by long term therapies due to lack of an effective antiviral agent against adenovirus.[14] In a clinical study, topical cidofovir, used alone or in combination with topical cyclosporine, did not accelerate improvement of clinical symptoms of acute adenoviral keratoconjunctivitis compared with the natural course of disease.[15] This shortcoming has led to the exploration and trial of other modalities of treatment.

The use of topical steroids to treat SEIs is controversial. They are frequently prescribed by eye care providers in acute phase, although this may only have a transient alleviating effect. The disease and infection durations could be prolonged because of increased adenovirus replication rate and extended viral shedding as demonstrated in animal model.[6] Second, steroids have the propensity to cause serious side effects like rise in IOP and development of cataract.

Topical 2% cyclosporine has been an alternative to treat subepithelial infiltrates in the acute phase of infection, but results, side effects and recurrence rates have been comparable to corticosteroids.[16]

This randomized, double masked trial compared the efficacy of 0.03% tacrolimus with 0.05% dexamethasone ointment for treating SEIs. The results suggest that there was a significant improvement ($P < 0.001$) in subjective symptoms, vision, and reduction in SEIs in both the treatment groups at 6 months. However, the magnitude of improvement in test parameters was higher in tacro group. Repeated measure ANOVA revealed that resolution of SEIs was quicker in tacro group (at three months of therapy) as compared to dexa group (after four months). Ghanem *et al*. applied 0.02% tacrolimus eye drops for the treatment of SEIs and observed a significant improvement in vision at final follow-up examination.[17] In the same way, Levinger *et al*. reported a significant improvement in the visual function of patients treated with 0.03% tacrolimus ointment after 18 weeks with a four-week wash-out period.[18]

The initial tolerability to the drug was significantly better in dexa group however, there was a significant rise in intraocular pressure; five (11.1%) out of 7 (15.6%) patients who had a sustained rise in IOP had to discontinue dexamethasone and shift to tacrolimus therapy. However, burning, redness and foreign body sensation were common with application of tacrolimus ointment and may be severe enough to discontinue treatment.

Subepithelial infiltrates have propensity to recur after cessation of therapy. Recurrence was more common (17.5%%) in dexa group and tapering of dose was unsuccessful after reintroduction of drug. In a study comparing topical loteprednol versus topical dexamethasone for treating SEIs after viral conjunctivitis, Kocluk *et al*. found that both groups were had substantial recurrence and the difference between groups (Loteprednol versus dexamethasone) was not significant.[19]

The recurrence rate for tacrolimus (7.5%) observed in the present study were lower than other studies. Better patient compliance, patient counselling and reduction in dose (once daily) instead of complete withdrawal of drug could probably account for lower recurrence rates. Prado *et al*. observed a recurrence rate of 18.8% for tacrolimus compounded in pharmacy.[20] The recurrence rate may be significantly high (12.5%) for topical cyclosporine 0.05% therapy.[21]

Conclusion

In conclusion, tacrolimus 0.03% ointment is more effective than dexamethasone 0.05% with low recurrence rate, no significant rise in IOP but may cause burning and foreign body sensation in some patients.

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Conflicts of interest

There are no conflicts of interest.

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