

Topical tacrolimus for the management of acute allergic conjunctivitis in a mouse model

Irina S. Barequet · Eva Platner · Kobi Sade · Sara Etkin · Hana Ziv · Mordechai Rosner · Zohar Habot-Wilner

Received: 27 September 2012 / Revised: 13 March 2013 / Accepted: 18 March 2013 / Published online: 5 April 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract

Background Acute allergic conjunctivitis is a constantly challenging condition that often requires steroids for effective management. Alternative treatment options are needed due to the potential side effects of steroids. Tacrolimus has been used for vernal/atopic conjunctivitis. The aim of our study was to investigate the therapeutic effect of topical administration of 0.03 % tacrolimus (eye drops or ointment) in comparison to 0.1 % dexamethasone in a mouse model of acute allergic conjunctivitis.

Methods BALB/c mice were sensitized by an intraperitoneal injection of 10 µg/0.2 ml ovalbumin (OVA) absorbed on ALUM (2.0 mg) on days 1 and 8. They were challenged by topical instillation of 2 µl of 15 % OVA (absorbed in 10 % glycerol) twice daily, on days 15–21. Treatment was administered twice daily on days 17–21. Mice were randomly assigned topical treatment groups: Group 1, 0.1 % dexamethasone drops; Group 2, 0.03 % tacrolimus drops; Group 3, 0.03 % tacrolimus ointment; Group 4 PBS drops (control). On day 22 all mice underwent clinical evaluation, blood sampling for IgE levels, and conjunctivas were removed for eosinophil counting.

Results IgE and OVA-specific IgE levels were similar among all groups, demonstrating induction of allergic reaction in all mice. Significantly lower clinical scores were found among all treated groups as compared to controls ($P < 0.001$), while no significant difference was found among the three treatment groups ($P > 0.05$). Conjunctival eosinophil counts were significantly lower in Group 1 ($P < 0.05$) as compared to the other groups.

Conclusions The clinical efficacy of topical 0.03 % tacrolimus was similar to 0.1 % dexamethasone for acute allergic conjunctivitis.

Keywords Allergic conjunctivitis · Tacrolimus · FK506

Introduction

Allergic conjunctivitis affects up to 25 % of the general population and is a common clinical problem for both ophthalmology and allergy practices [1, 2]. The most characteristic symptom is ocular itching, followed by burning sensation, photophobia and tearing. The common clinical signs are conjunctival redness, tearing, mucus discharge, chemosis and lid edema.

The common form of allergic conjunctivitis is the immediate type that presents as seasonal and perennial allergic conjunctivitis [3]. This type is caused by IgE-mediated response and typically elicited by the ocular exposure to an allergen. The allergen must be recognized and presented to Langerhans cells, which present the antigen to T lymphocytes. T lymphocytes are divided into CD4+ T-helper (Th1, Th2) and CD8+ killer T cells. In the immediate type allergic reaction, activated Th2 cells regulate the maturation of B lymphocytes to mature IgE antibody-producing B cells and plasma cells. The allergen reacts with specific IgE antibodies bound to the

Irina S. Barequet and Eva Platner contributed equally to the work presented here and should therefore be regarded as equivalent authors.

I. S. Barequet (✉) · E. Platner · H. Ziv · M. Rosner
Goldschleger Eye Institute, Sheba Medical Center, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Hashomer 52621, Israel
e-mail: ibarequet@hotmail.com

K. Sade · S. Etkin
Allergy and Asthma Center, Tel Aviv Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Z. Habot-Wilner
Department of Ophthalmology, Tel-Aviv Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

surface of conjunctival mast cells. The activation of mast cells triggers mast cell degranulation with the release of chemotactic factors that are responsible for accumulation of eosinophils and neutrophils at the site of allergic inflammation. Eosinophils contain granules in which preformed cytokines are rapidly secreted. Hence, a cascade of allergic and inflammatory mediators, such as histamine, cytokines and prostaglandins is produced [2, 4, 5].

The mainstay of therapy for allergic conjunctivitis includes topical H1-histamine receptor antagonists and mast cell stabilizers [3]. However, in severe or recalcitrant cases the use of corticosteroids is needed. Due to the potentially serious side effects of topical corticosteroids, such as elevated intraocular pressure, cataract formation or progression and increased risk of opportunistic infections, there is a need for a safe and efficient alternative treatment.

Tacrolimus (FK506) is a macrolide immunosuppressive agent isolated from the bacterium *Streptomyces tsukubaensis*. It inhibits the activation of T lymphocytes by binding to the cytosolic protein FK506-binding protein-12 [6, 7], thus inhibiting activation of the genes of interleukin (IL)-2, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor, interferon, and other ILs that are involved in the regulation of an immune response (delayed-type hypersensitivity) [8–10]. In addition, tacrolimus inhibits the release of histamine from mast cells and impairs prostaglandin synthesis in its de novo way [11]. These actions together may reduce the allergic reaction.

Several clinical studies have been published on the use of tacrolimus for various ocular allergic diseases. Tacrolimus was found useful for severe vernal keratoconjunctivitis [12, 13], severe allergic conjunctivitis [14], intractable allergic conjunctivitis [15] and atopic blepharoconjunctivitis [16–19]. In addition, in an experimental allergic conjunctivitis mouse model it was shown that topical dexamethasone and tacrolimus treatments were both efficacious in suppressing eosinophil and lymphocyte infiltration into subconjunctival tissue [20]. However, no study examined the efficacy of tacrolimus in the treatment of acute allergic conjunctivitis.

The aim of our study was to investigate the therapeutic effect of topical administration of tacrolimus (eye drops or ointment) in comparison to dexamethasone in a mouse model of acute allergic conjunctivitis.

Materials and methods

Animals

Experiments were carried out in accordance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by the Sackler Faculty of

Medicine, Tel Aviv University Institutional Animal Care and Use Committee.

Female BALB/c mice were housed under a 12-h light–dark cycle (light on from 07:00 to 19:00), with room temperature maintained at 18–20 °C, humidity at 40–60 % and an allergen free environment. Food and water were given ad libitum.

Reagents

The sensitization and challenge agents included Ovalbumin (OVA) and Aluminium hydroxide (ALUM) (Sigma-Aldrich Ltd, Israel).

The therapy agents included 0.03 % tacrolimus eye drops (Vetmarket Marketing Ltd., Israel), 0.03 % tacrolimus ointment (Protopic: Astellas Pharma Europe B.V, The Netherlands), 0.1 % dexamethasone sodium phosphate eye drops (Sterodex, Dr. Fisher, Israel) and PBS (phosphate buffered saline).

Experimental allergic conjunctivitis, treatment and assessment

Mice were actively sensitized by an intraperitoneal injection of 0.2 mL of 10 µg ovalbumin (OVA) absorbed on ALUM (2.0 mg) on days 1 and 8. They were challenged by topical instillation of 2 µL of 15 % OVA (absorbed in 10 % glycerol) twice daily, on days 15–21.

On days 17–21 all mice were treated twice daily according to the randomly assigned treatment groups. Group 1 received topical 0.1 % dexamethasone drops, group 2 received 0.03 % tacrolimus drops, group 3 received 0.03 % tacrolimus ointment, and group 4 (control) received PBS drops. On day 22 all mice underwent clinical assessment by slit lamp biomicroscopy. Blood was drawn for IgE levels and the animals were sacrificed. Upper lid, lower lid and the eyeball were removed for histopathological examination.

Clinical grading

On day 22, each eye underwent slit lamp evaluation by a masked, independent observer for lid swelling, discharge, conjunctival injection and conjunctival chemosis. Each of the four parameters was graded from 0 to 3 according to severity (0- no finding, 1- mild, 2- moderate, 3 severe). The grades of each of the four parameters were added to obtain a total score (0–12).

IgE level measurements

Twelve hours after the last treatment dose (day 22) blood was drawn for evaluation of IgE and OVA-specific IgE

levels. The levels were determined by enzyme-linked immunosorbent assay (ELISA), using the mouse IgE ELISA Quantitation Set (Bethyl laboratories inc., Cat no. E90-115).

Histological analysis

All upper lids, lower lids and eyeballs including the bulbar conjunctiva were fixed in 10 % neutral buffered formalin solution and embedded in paraffin. Vertical sections (thickness, 4 mm) were cut. Samples were stained with hematoxylin & eosin. Fields from each eye including the palpebral and bulbar conjunctiva at magnification of X400 were photographed using photomicroscope (OLYMPUS DP71 digital cameras, Spach Optics Inc.). Eosinophils were counted in a masked fashion from the pictures using ImageJ software (<http://rsbweb.nih.gov/sheba-ez.medlcp.tau.ac.il/ij/>).

Statistical analysis

The means of data are presented with SEM. Data from 4 groups were statistically evaluated using One-way ANOVA. The analysis was based on the root mean square, in order to obtain a normal distribution. Wherever significant differences between the groups were found, the Tukey's test for multiple comparisons was applied. $P < 0.05$ was considered statistically significant.

Results

The study included 51 mice that were randomly assigned to the four treatments groups: Group 1 ($n=13$) received 0.1 % dexamethasone eye drops, Group 2 ($n=13$) received 0.03 % tacrolimus eye drops, Group 3 ($n=12$) received 0.03 % tacrolimus ointment, and Group 4 (control group, $n=13$) received PBS.

IgE level measurements

IgE and OVA-specific IgE levels were found similar among all groups (Table 1). These findings demonstrate the induction of similar allergic sensitization in all groups. The levels

of the OVA-specific IgE of the not-sensitized, untreated mice were undetectable.

No correlation was found between the serum levels of IgE or OVA-specific IgE and the clinical signs, using Pearson correlation test.

Clinical evaluation

The clinical evaluation at the conclusion of the study (day 22, i.e., 12 h after the last treatment application) showed significantly lower scores among all treated groups (group 1: 0.15 ± 0.28 , group 2: 0.15 ± 0.26 , group 3: 0.41 ± 0.55) as compared to the control (2.30 ± 0.57) ($P < 0.001$). No significant difference was found among the three treatment groups ($P > 0.05$, Fig. 1).

Histological analysis

The total conjunctival eosinophil counts were: Group 1 (24.1 ± 16.4), Group 2 (78.2 ± 69.8), Group 3 (110.6 ± 85), and Group 4 (124.3 ± 118). A statistically significant difference was found only in Group 1 ($P < 0.05$). Figure 2 shows the eosinophil infiltrations in the four treatment groups.

Discussion

In this study we induced an acute allergic reaction by sensitizing BALB/c mice with ovalbumin (OVA). We assessed the effect of topical tacrolimus (eye drops or ointment) and dexamethasone eye drops on the clinical findings and the eosinophil infiltration in the histological sections of the conjunctivas. The dexamethasone treatment served as a gold standard, and PBS served as control. We administered tacrolimus as eye drops and as ointment in order to compare the efficacy of different formulations of the same concentration (0.03 %). The allergic model was achieved in all mice as supported by the presence of similar levels of IgE and OVA-specific IgE. We found that 0.03 % tacrolimus eye drops, 0.03 % tacrolimus ointment, and 0.1 % dexamethasone eye drops - all showed a significant effect in suppressing the clinical signs of the allergic reaction as compared to PBS. Although the clinical scores were lower in the tacrolimus eye drops group than in the

Table 1 IgE and OVA-specific IgE blood levels in the various treatment groups

Average IgE ^a level	Group 1 Dexamethasone 0.1 % eye drops	Group 2 Tacrolimus 0.03 % eye drops	Group 3 Tacrolimus 0.03 % ointment	Group 4 PBS ^b (Control)	P value
IgE (ng/ml)	266±100 ($n=11$)	197±133 ($n=13$)	229±143 ($n=10$)	247±153 ($n=13$)	0.63
OVA ^c -specific IgE (ng/ml)	27±12 ($n=11$)	16±7 ($n=11$)	20±12 ($n=9$)	18±12 ($n=12$)	0.11

^a immunoglobulin E, ^b phosphate buffered saline, ^c ovalbumin

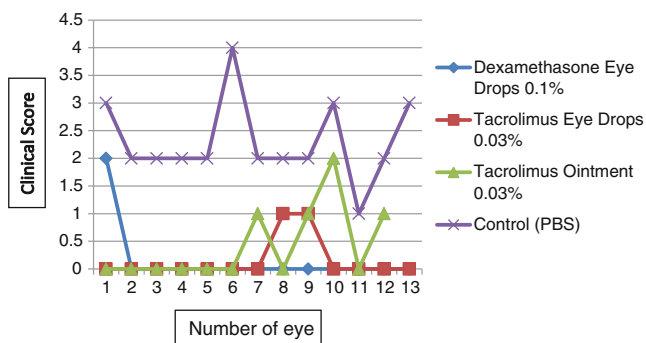


Fig. 1 The mean clinical scores at the conclusion of treatment. *A significant difference was found between each of the 3 treatment groups and the control group ($P < 0.001$), and no significant differences were found among the three treatment groups ($P > 0.05$)

tacrolimus ointment group, this difference did not reach a statistical significance. The histological findings showed smaller eosinophil counts among all treatment groups than in the control groups, however only the dexamethasone 0.1 % reached statistical significance.

Although acute allergic conjunctivitis is initially induced through immediate-type responses triggered by mast cells sensitized with IgE, a prolonged conjunctival inflammation occurs as well via a late response, as evident from the infiltration of inflammatory cells such as eosinophils, neutrophils and T-helper 2 into the conjunctiva [21].

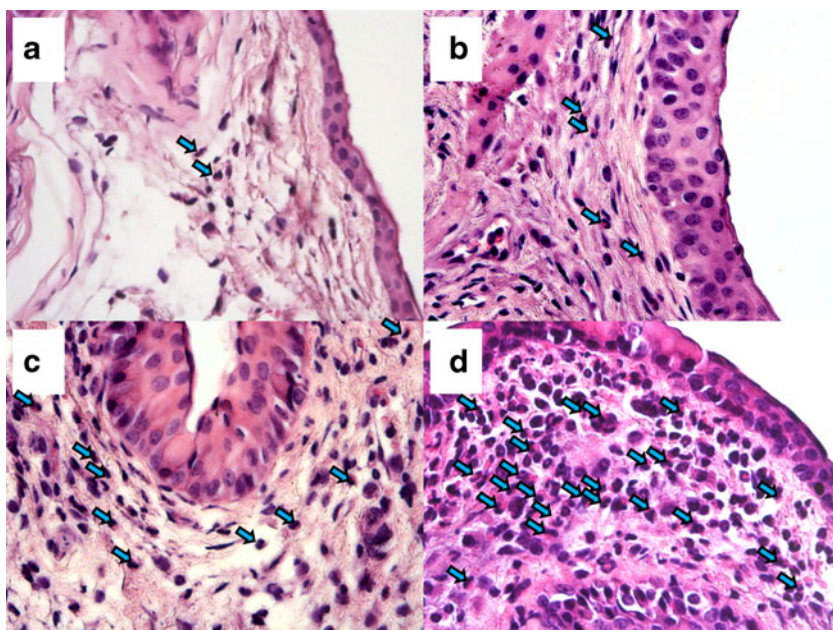
In the clinical setting, the treatment of acute allergic conjunctivitis is frequently challenging due to limited response to currently available topical H1-histamine receptor antagonists and mast cell stabilizers. In these cases, topical corticosteroids are needed to suppress the allergic reaction; however this bears potentially serious ocular adverse effects [22]. Tacrolimus

(FK506) inhibits the release of histamine from mast cells and impairs prostaglandin synthesis which are responsible for the acute allergic reaction, in addition to suppressing eosinophil infiltration and regulating the delayed-type hypersensitivity immune reaction [8–11, 20].

Our study is the first to assess the effect of tacrolimus in the setting of an acute allergic conjunctivitis model, and showed that topical tacrolimus was as effective as dexamethasone in improving the clinical signs. Despite the significant clinical effect, the histological analysis showed less eosinophils counts among the tacrolimus-treated eyes, however this decrease was not statistically significant. Shoji and colleagues [20] demonstrated that both topical 0.1 % dexamethasone ointment and 0.1 % tacrolimus ointment were efficacious in suppressing eosinophil and lymphocyte infiltration into subconjunctival tissue, as prophylaxis in a mouse experimental allergic conjunctivitis model. We used 0.03 % topical tacrolimus, which is commercially available both as ointment and eye drops, but is lower than the 0.1 % tacrolimus ointment that was administered in their study. Moreover, histological assessment of eosinophils may not reflect the entire therapeutic response to tacrolimus, as other inflammatory mediators are also involved in the allergic process and altogether may influence the clinical findings. Additional studies on inflammatory mediators in the conjunctiva could elaborate the efficacy of topical tacrolimus in the setting of acute allergic reaction.

In summary, this study showed that the clinical efficacy of topical 0.03 % tacrolimus was similar to 0.1 % dexamethasone for acute allergic conjunctivitis. Topical tacrolimus may be considered as an alternative treatment to dexamethasone when topical steroids are contraindicated.

Fig. 2 Microscopic photographs of the conjunctiva 12 h after the last treatment application (haematoxylin and eosin stain, magnification of X400). The light blue arrows indicate eosinophils. a. 0.1 % dexamethasone eye drops, b. 0.03 % tacrolimus eye drops, c. 0.03 % tacrolimus ointment, d. PBS (control). Not all eosinophils are marked by arrows



Financial support This study was supported by a grant from the Claire and Amedee Maratier Eye Institute for the Study of Blindness and Visual Disorders, Tel Aviv University.

All the authors have full control of all primary data and agree to allow Graefes Archives for Clinical and Experimental Ophthalmology to review the data upon request.

References

- Kay AB (2001) Allergy and allergic diseases. First of two parts. *N Engl J Med* 344:30–37
- Ono SJ, Abelson MB (2005) Allergic conjunctivitis update on pathophysiology and prospects for future treatment. *J Allergy Clin Immunol* 115:118–122
- Kari O, Saari KM (2012) Diagnostics and new developments in the treatment of ocular allergies. *Curr Allergy Asthma Rep* 12:232–239
- Spencer LA, Szela CT, Perez SA, Kirchhoffer CL, Neves JS, Radke AL, Weller PF (2009) Human eosinophils constitutively express multiple TH1, Th2 and immunoregulatory cytokines that are secreted rapidly and differentially. *J Leukoc Biol* 85:117–123
- Offiah I, Calder VL (2009) Immune mechanisms in allergic eye diseases: what is new? *Curr Opin Allergy Clin Immunol* 9:477–481
- Harding MW, Galat A, Uehling DE, Schreiber SL (1989) A receptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase. *Nature* 341:758–760
- Siekierka JJ, Hung SH, Poe M, Lin CS, Sigal NH (1989) A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. *Nature* 341:755–777
- Ruff VA, Leach KL (1995) Direct demonstration of NFATp dephosphorylation and nuclear localization in activated HT-2 cells using a specific NFATp polyclonal antibody. *J Biol Chem* 270:22602–22607
- Tamura K, Fujimura T, Iwasaki K, Sakuma S, Fujitsu T, Nakamura K, Shimomura K, Kuno T, Tanaka C, Kobayashi M (1994) Interaction of tacrolimus (FK506) and its metabolites with FKBP and calcineurin. *Biochem Biophys Res Commun* 202:437–443
- Liu J, Farmer JD Jr, Lane WS, Friedman J, Weissman I, Schreiber SL (1991) Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* 66:807–815
- Ruzicka T, Assmann T, Homey B (1999) Tacrolimus: the drug for the turn of the millennium? *Arch Dermatol* 135:574–580
- Vichyanond P, Tantimongkolsuk C, Dumrongkigchaiporn P, Jirapongsananuruk O, Visitsunthorn N, Kosrirukvongs P (2004) Vernal keratoconjunctivitis: result of a novel therapy with 0.1 % topical ophthalmic FK-506 ointment. *J Allergy Clin Immunol* 113:355–358
- Kheirkhah A, Zavareh MK, Farzod F, Mahbod M, Behrouz MJ (2011) Topical 0.005% tacrolimus eye drop for refractory vernal keratoconjunctivitis. *Eye (Lond)* 25:872–880
- Ohashi Y, Ebihara N, Fujishima H, Fukushima A, Kumagai N, Nakagawa Y, Namba K, Okamoto S, Shoji J, Takamura E, Hayashi K (2010) A randomized, placebo-controlled clinical trial of tacrolimus ophthalmic suspension 0.1 % in severe allergic conjunctivitis. *J Ocul Pharmacol Ther* 26:165–167
- Attas-Fox L, Barkana Y, Iskhakov V, Rayvich S, Gerber Y, Morad Y, Avni I, Zadok D (2008) Topical tacrolimus 0.03 % ointment for intractable allergic conjunctivitis: an open-label pilot study. *Curr Eye Res* 33:545–549
- Rikkers SM, Holland GN, Drayton GE, Michel FK, Torres MF, Takahashi S (2003) Topical tacrolimus treatment of atopic eyelid disease. *Am J Ophthalmol* 135:297–302
- Nivenius E, van der Ploeg I, Jung K, Chrysanthou E, van Hage M, Montan PG (2007) Tacrolimus ointment vs steroid ointment for eyelid dermatitis in patients with atopic keratoconjunctivitis. *Eye (Lond)* 21:968–975
- Zribi H, Descamps V, Hoang-Xuan T, Crickx B, Doan S (2009) Dramatic improvement of atopic keratoconjunctivitis after topical treatment with tacrolimus ointment restricted to the eyelids. *J Eur Acad Dermatol Venereol* 23:489–490
- Virtanen HM, Reitamo S, Kari M, Kari O (2006) Effect of 0.03 % tacrolimus ointment on conjunctival cytology in patients with severe atopic blepharoconjunctivitis: a retrospective study. *Acta Ophthalmol Scand* 84:693–695
- Shoji J, Sakimoto T, Muromoto K, Inada N, Sawa M, Ra C (2005) Comparison of topical dexamethasone and topical FK506 treatment for the experimental allergic conjunctivitis model in BALB/c mice. *Jpn J Ophthalmol* 49:205–210
- Bonini S, Bonini S, Vecchione A, Naim DM, Allansmith MR, Balsano F (1988) Inflammatory changes in conjunctival scrapings after allergen provocation in humans. *J Allergy Clin Immunol* 82:462–469
- Mandal R, Maiti P, Sasmal NK, Sinha N, Gupta A, Das KS, Biswas MC (2011) Ocular effects of long term use of topical steroids among children and adolescents with vernal keratoconjunctivitis: a prospective observational study. *J Indian Med Assoc* 109(708–10):712–713