H. Iwamoto
H. Yoshida
O. Yoshida
A. Fukushima
H. Ueno

Inhibitory effects of FK506 on the development of experimental allergic/immune-mediated blepharoconjunctivitis in Lewis rats by systemic but not by topical administration

Received: 28 October 1997

Revised version received: 16 January 1998

Accepted: 26 February 1998

Abstract ● Background: FK506 has been used for treatment of cell-mediated immune disorders such as graft rejection in transplantation or Behçet disease. To evaluate the effectiveness of FK506 in another ocular disease model, we injected FK506 in rats with experimental allergic/immune-mediated blepharo conjunctivitis (EAC) the induction mechanism of which depends on cell-mediated immunity. ● Methods: Lewis rats were immunized with ovalbumin (OVA) in emulsion of complete Freund's adjuvant (CFA). We injected 2 ($n=6$), 20 ($n=6$) or 200 ($n=5$) μg of FK506 intramuscularly daily from the day of immunization (day 0) to day 6. Control rats were not treated with FK506 ($n=4$). In addition, we injected 200 μg of FK506 from day 7 to day 13 ($n=12$) to compare the timing of FK506 administration (day 0 to day 6, $n=12$; control, $n=12$). Twenty-one days after immunization, all rats were challenged with OVA by eye drops, and 24 h later they were killed after clinical evaluation and their eyes, blood and draining lymph nodes were harvested for histology, antibody titers and proliferation assay or flow cytometric analysis. In another set of experiments, rats that had received OVA-primed lymph node cells did

($n=9$) or did not ($n=9$) receive additional FK506 by injection daily for 4 days. Four days after transfer, these rats were challenged with OVA and evaluated as mentioned. To investigate possible suppression of disease by topical administration of FK506, both actively immunized and passively immunized rats received OVA together with 0.3% (weight/volume) of FK506 ($n=16$) or vehicle ($n=10$) by eye drops and 24 h after challenge, rats were evaluated as mentioned. ● Results: Development of disease, induced by either active or passive immunization, was inhibited in the group treated with 200 μg of FK506, regardless of timing of administration. Cellular proliferative responses to OVA were inhibited only in this group. Flow cytometry demonstrated a decrease of about 20% in the proportion of all cells made up by CD4-positive T cells. Topical administration of FK506 inhibited the development of EAC, though not significantly. ● Conclusions: Systemic treatment with 200 μg of FK506 either in the induction or the effector phase inhibits the development of EAC in Lewis rats. Topical administration is not so effective as systemic administration.

H. Iwamoto · H. Yoshida · O. Yoshida
A. Fukushima (✉) · H. Ueno
Laboratory of Immunology,
Department of Ophthalmology,
Kochi Medical School, Kohasu,
Okochi-cho, Nankoku 783–8505, Japan

Introduction

Allergic conjunctivitis is a group of diseases characterized by hypersensitive immune-mediated inflammation against exogenous antigens in the conjunctiva [6]. Clinical and experimental reports have demonstrated that IgE-mediated immediate responses are dominant mechanisms for the development of this disease [5, 15]. Although humoral immunity is the major effector mechanism for the development of allergic conjunctivitis, cellular immunity is also thought to be involved in the development of certain kinds of allergic conjunctivitis such as vernal keratoconjunctivitis (VKC) [1, 3]. Recently, we demonstrated the essential role of cellular immunity for the induction of experimental allergic/immune-mediated blepharoconjunctivitis (EAC) in Lewis rats [8]. The histopathological features of EAC are similar to those of VKC. In our system, EAC was induced either by active immunization or adoptive transfer of allergen-primed lymph node cells.

FK506 is known to suppress cellular immunity by inhibiting production of cytokines and IL-2 receptor expression of T cells, leading to reduction in number and function of CD4-positive T cells [2, 18]. FK506 exerts its effects at 10–100 times lower concentrations than cyclosporin A (CsA) [19]. Therefore, it has been used both in experimental animals and in humans for treatment of cell-mediated immune disorders such as graft rejection in organ transplantation [20, 21].

Concerning eye diseases, Mochizuki et al. extensively studied the effects of systemic administration of FK506 in experimental autoimmune uveitis [7, 12] and in patients with uveitis [17]. In addition, they investigated the effect of topical application of FK506 or CsA on endotoxin-induced uveitis [9] or corneal allograft transplantation [10] and demonstrated the effectiveness in terms of suppression of development of disease.

For the treatment of allergic conjunctivitis, anti-histamine drugs are the first choice, and topical steroid therapy is selected for severe cases [11]. Recently, however, immunosuppressive drugs targeted to T cells, such as cyclosporin A or FK506, were discovered, and trials of topical administration demonstrated that these drugs were just as effective as corticosteroids [11]. In addition to allergic conjunctivitis, treatment of FK506 effectively suppressed the development of allergic diseases such as contact dermatitis [13, 14]. These reports prompted us to investigate the inhibitory effects of either systemic or topical administration of FK506 on EAC in Lewis rats, in which cellular immunity is dominantly involved.

Materials and methods

Rats

Six- to 8-week-old male Lewis rats were purchased from a commercial source (Seac Yoshitomi, Fukuoka, Japan) and maintained in a

specific pathogen-free animal facility at Kochi Medical School. All animal procedures conformed to institutional guidelines and the ARVO Resolution on Use of Animals in Research.

Reagents

FK506 was provided by Fujisawa Pharmaceutical, Osaka, Japan. For systemic administration by intramuscular injection, FK506 was suspended in phosphate-buffered saline (PBS) to achieve the required concentrations. For topical administration by eye drops, 0.3% (weight per volume) of FK506 and vehicle were prepared by the company.

Immunization

Rats were injected with 100 µg of ovalbumin (OVA; grade V, Sigma Immunochemicals, St. Louis, Mo.) emulsified with complete Freund's adjuvant (CFA; Yatoron, Tokyo, Japan) in a final volume of 100 µl in their left hind footpads.

Experimental protocol

Active immunization (dose-response)

The day of immunization was regarded as day 0. Tested groups of rats received 2 µg ($n=6$), 20 µg ($n=6$) or 200 µg ($n=5$) of FK506 daily from day 0 to day 6 by intramuscular injection. Control rats were not treated with FK506. Twenty-one days after immunization, all rats were challenged with OVA by eye drops as previously reported. Twenty-four hours later, they were clinically evaluated, killed, and eyes, including lids, blood, and draining lymph nodes, were harvested for histology, ELISA, proliferative responses and flow cytometry.

Active immunization (timing for FK506 administration)

Procedures were the same as described above except for the timing of FK506 injection. Tested groups of rats received 200 µg of FK506 daily from day 0 to day 6 (early induction phase; group 1, $n=12$), and from day 7 to day 13 (late induction phase; group 2, $n=12$). Group 3 were control rats without FK506 treatment ($n=12$).

Passive immunization by adoptive transfer of OVA-primed lymph node cells (systemic administration)

Cells from immune inguinal lymph nodes were harvested 14 days after immunization. Cells were washed three times and then stimulated with OVA for 3 days as detailed in our previous report [8]. 30×10^6 stimulated cells were injected into syngeneic male Lewis rats (recipients) by intravenous injection. Tested groups of rats received daily intramuscular injections of 200 µg of FK506 from the day of transfer to the day of challenge ($n=9$; control $n=9$). Four days after transfer, recipients were challenged with OVA by eye drops; 24 h later, the same procedures as described above [Active immunization (dose-response)] were performed.

Passive immunization by adoptive transfer of OVA-primed lymph node cells (topical administration)

EAC was induced either by active or passive immunization of cell transfer following the same procedures as above. Tested groups of rats received 10 µl of FK506 by eye drops. Twenty-four hours before challenge and 1, 2, 3, 4, 6, and 12 h after challenge, they received

Fig. 1 a Clinical grading of EAC (Dose-response of FK506). Tested groups of rats received 2 µg, 20 µg, or 200 µg of FK506 daily from day 0 to 6 by intramuscular injection. Control rats were not treated with FK506. Each dot indicates an individual rat. Clinical grading was scored following Materials and methods

Fig. 1 b Histology of the conjunctiva of rats with EAC either untreated or treated with FK506. Above: not treated at all; below: treated with 200 µg of FK506 from day 0 to day 6. Rats were actively immunized with OVA and challenged with OVA by eye drops as described in Materials and methods. Magnification $\times 320$

10 µl of FK506 by eye drops ($n=16$). Control rats were treated with vehicle by eye drops ($n=10$). Twenty-four hours later, all rats were clinically evaluated and killed for histological study.

Clinical grading and histological studies of conjunctiva

The clinical scoring system used was based on our previous report [8]. In brief, each eye was assessed for the findings of lid swelling and discharge, and each finding was scored as one point. Ocular changes were also examined histologically. After clinical evaluation, eyes, including the lids, were fixed in 10% buffered formalin and embedded in paraffin. Ten-micrometer sections were cut and stained with hematoxylin and eosin. All the infiltrated cells which we could not categorize as macrophages or lymphocytes by this staining were counted in the palpebral conjunctiva per field, using an eye-piece grid at 400 $\times$ magnification. The data were represented as cells/mm². By this method, less than 5 cells/mm² were counted in the normal conjunctiva of the rat.

Proliferative responses of draining lymph node cells

Lymph nodes or spleens from two or three rats were combined in each group and experiments were repeated as recorded. Lymph node

cells were used for experiments of active immunization, splenocytes for those of passive immunization. Lymphocyte proliferation assays were set up in quadruplicate in 96-well flat-bottom plates. Either lymph node cells or splenocytes (3×10^5 cells/well) were cultured in a final volume of 0.2 ml RPMI 1640 medium supplemented with 5% FCS, 2-ME (5×10^{-5} M), L-glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 µg/ml). Cells were stimulated with OVA at final concentrations of 0.1, 1, 10, or 100 µg/ml or with concanavalin A (Con A; Sigma Immunochemicals, St. Louis, Mo.) at 1 µg/ml or purified protein derivative (PPD, Japan BCG, Tokyo, Japan) at 1 µg/ml. After incubation for 72 h at 37°C in a humidified atmosphere with 5% CO₂, cultures were pulsed for 16 h with 0.5 µCi/well of ³H-thymidine (Japan Atomic Energy Research Institute, Tokai, Japan). Cultures were then harvested and the radioactivity was measured by standard techniques. Data were expressed as stimulation indices.

Anti-OVA antibody titers of serum from rats

Serum was collected from each rat via cardiac puncture at the time of death. Serum antibody levels against OVA were measured by direct ELISA. OVA (500 ng/well) were adsorbed to 96-well plates for 2 h at 37°C. The plates were washed with PBS-Tween, and serially diluted serum samples were added to the wells. Bound antibody was detected by peroxidase-conjugated anti-IgG (Nordic Immunological Laboratories, Tilburg, The Netherlands). The plates were developed using 3,3',5,5'-tetramethylbenzidine base (TIMB-ELISA; Gibco BRL, Gaithersburg, Md.), and the optical density (OD) for each well was read at 610 nm.

Flow cytometry

Cells were stained with following anti-rat antibodies (Abs). Anti-CD4 and anti-CD8 (Pharmingen, San Diego, Calif.) are FITC labeled. Anti-CD3 and anti-CD25 (Pharmingen), anti-I-A (OX-3) and anti-CD45RA (OX-33) (Serotec, Oxford, UK), and anti-CD11a (LFA-1α) (Caltag Laboratories, Burlingame, Calif.) are biotin-labeled. The cells were incubated for 45 min on ice with the primary Abs above (0.02 – 2 µg/ 2×10^6 cells) and washed with cold 5% FCS-PBS. Streptavidin-PE (Pharmingen) was added to cells stained with biotin-conjugated Abs for 10 min and then the cells were washed again. The cells were then analyzed using FACScan (Becton Dickinson, Mountain View, Calif.).

Statistical analysis

Statistical comparison of the number of infiltrated cells between two groups was performed using Student's *t*-test.

Table 1 Effects of systemic Fk506 treatment on cellular immunological parameters

Treatment ^a	Average cells recovered ^b	Proliferation against ^c			Number of tested rats
		OVA (100 µg/ml)	Con A (1 µg/ml)	PPD (1 µg/ml)	
None	64.8×10^6	17380	74314	4673	4
2 µg	51.0×10^6	22494	105187	6391	6
20 µg	75.5×10^6	15695	60006	5406	6
200 µg	58.2×10^6	1479	56120	852	5

^a Treatment was done from day 0 to day 6 as described in Materials and methods

^b Total recovered cell number from left inguinal lymph nodes divided by number of rats

^c Proliferation assay performed as described in Materials and methods, data presented as delta cpm (cpm in presence of tested antigen–cpm without antigens)

Results

Systemic FK506 treatment: dose response

To test the dose required to inhibit the induction of EAC, we injected three different doses of FK506 (2 µg, 20 µg and 200 µg) from day 0 to day 6 intramuscularly. As shown in Fig. 1, only daily administration of 200 µg FK506 was effective for suppression of EAC. This result was in accordance with the finding that lymph node cells of rats treated with 200 µg of FK506 exhibited lower proliferative responses than those of other groups. Cellular immunity against OVA in the groups of rats treated with 2 or 20 µg FK506 were comparable to the control group (Fig. 3a). There was no clear difference in number of lymph node cells recovered from each rat among all the groups (Table 1). Although there were no remarkable differences in the proliferative responses for Con A among these four groups of rats, lymph node cells from rats treated with 200 µg of FK506 exhibited diminished proliferative responses against PPD compared to those from other groups (Table 1).

Fig. 2 Clinical grading of EAC (timing of FK506 injection). Tested groups of rats received 200 µg of FK506 daily from day 0 to 6 (Group 1) or from day 7 to 13 (Group 2) by intramuscular injection. Control rats were not treated with FK506 (Group 3). Each dot indicates an individual rat. Clinical grading was scored following Materials and methods

Fig. 3 **a** Proliferative responses of draining lymph node cells against OVA (dose-response of FK506). Draining lymph node cells from two to three rats were combined and tested for proliferation as detailed in Materials and methods. The recorded data are means of stimulation index (S.I.) values. Similar results were obtained in another experiment. The combined mean cpm $\pm$ SE values in the unstimulated control cultures were: none: 1115 $\pm$ 47; 2 μ g of FK506: 2591 $\pm$ 134; 20 μ g of FK506: 1233 $\pm$ 107; 200 μ g of FK506: 897 $\pm$ 16. **b** Proliferative responses of draining lymph node cells against OVA (timing of FK506 injection). Draining lymph node cells from two to three rats were combined and tested for proliferation detailed in materials and methods. The recorded data are means of stimulation index values. Similar results were obtained in three

Table 2 Data expressed as proportion of total cells tested that were positive (%)

Treatment	CD3	CD4	CD8	CD45RA	LFA-1
None	64.4	50.5	22.7	27.3	99.2
FK506 ^a	46.2	32.3	22.3	48.4	99.3

^a 200 μ g of FK506 injected intramuscularly from day 0 to day 6

Systemic FK506 treatment on EAC: comparison of timing of administration

To evaluate the influence of the timing of FK506 administration of the inhibition of the induction of EAC, we injected 200 μ g of FK506 intramuscularly daily, either from day 0 to day 6 or from day 7 to day 13. No animals except for one rat in group 1 and one in group 2 showed any clinical pathologic changes. In contrast, almost all of the control rats had discharge and/or lid swelling (Fig. 2). Compared to the control group (group 3), both proliferative responses (Fig. 3a, b) and IgG production specific to OVA (Fig. 3c) were inhibited in group 1 and group 2. Immune responses against OVA were profoundly suppressed in group 1 compared to group 2 (Fig. 3b, c).

Flow-cytometric analysis of lymph node cells of FK506-treated rats

Lymph node cells from group 1 and group 3 were tested for their surface markers by FACscan analysis. As shown in Table 2, a 20% decrease was detected in both CD3- or CD4-positive cells in the FK506-treated group compared to the control group. In contrast, percentages of cells positive for CD45RA, which is a B cell marker, relatively increased. Taking the data of Table 1 into consideration, FK506 inhibited the increase of CD4-positive T cells in vivo, while B cells were not affected.

Systemic FK506 treatment inhibited EAC by adoptive transfer of OVA-pimmed lymph node cells

To investigate the effects of FK506 on the effector phase of EAC, we injected 200 μ g of FK506 each day from the day of transfer to the day of OVA challenge. Cellular infiltration was significantly suppressed ($P < 0.001$) by treatment with 200 μ g of FK506 (Fig. 4a). Proliferative re-

more independent experiments. The combined mean cpm $\pm$ SE values in the unstimulated control cultures were: group 1: 2348 $\pm$ 288; group 2: 7019 $\pm$ 312; group 3: 2135 $\pm$ 138. **c** Measurement of OVA-specific IgG by ELISA in serum from rats. Serum was collected at the time of death and assayed for ELISA. The X-axis indicates dilution factors of serum, while the Y-axis depicts absorbance at 610 nm. Similar results were obtained in two more independent experiments

Fig. 4 **a** Infiltrated cell number in the conjunctiva of recipients of OVA-primed cells either untreated or treated with systemic FK506. The infiltrated cells were counted in the palpebral conjunctiva per field and the bar indicates standard deviation. The X-axis indicates the number of infiltrated cells/mm². **b** Infiltrated cell number in the conjunctiva of recipients of OVA-primed cells either untreated or treated with topical FK506. The infiltrated cells were counted in the palpebral conjunctiva per field and the bar indicates standard deviation. The X-axis indicates the number of infiltrated cells/mm².

sponse of splenocytes from recipients treated with FK506 was also inhibited compared to the control rats (Fig. 5).

Topical FK506 treatment by eye drops partially suppressed the severity of EAC

The data presented above show that systemic treatment with FK506 was effective for suppression of EAC. We tested the possibility of suppression of EAC by treatment with topical FK506 in the form eye drops. In this study, we used both active immunization and passive immunization by adoptive transfer of OVA-primed lymph node

Fig. 5 Proliferative responses of splenocytes from recipients of OVA-primed cells. Splenocytes from two rats were combined and tested for proliferation as detailed in Materials and methods. The recorded data are means of stimulation index values of two rats from each group. Similar results were obtained in two more independent experiments. The combined mean cpm $\pm$ SE values in the unstimulated control cultures were: FK506-treated: 75 $\pm$ 3; no treatment: 177 $\pm$ 31

cells. The number of cells infiltrated, induced by either active immunization (data not shown) or passive immunization (Fig. 4b), was diminished, though not significantly so ($P=0.06$).

Discussion

The data recorded here demonstrated the inhibitory effects of FK506 administration on the development of EAC using both active and passive immunization systems. Although the nature of the target antigens (autoantigen versus exogenous antigen) differed between EAU and EAC in Lewis rats, the inducing mechanism of both diseases, which is mediated by cellular immunity [8, 16], is thought to be similar, because transfer of CD4-positive T cell line specific to OVA 323–339 peptide induced EAC after challenge with OVA in the form of eye drops (unpublished results). As injection of FK506 effectively inhibited the development of EAU [12], we tested the possible effects of FK506 on the suppression of EAC.

The required dose for suppression of EAC by systemic treatment during early induction phase was 200 μ g (Fig. 1a) the same dose as previously reported in the system of EAU in Lewis rats [12]. Dose dependency was observed in the suppression of disease as well as the inhibition of immune responses (Figs. 1a, 3a). As shown in Ta-

ble 1, 200 µg of FK506 suppressed the proliferative responses of lymph node cells against PPD as well as OVA, while it did not affect the proliferation of lymph node cells to Con A. These data suggest that FK506 induced non-antigen-specific immunosuppression and that this extensive immunosuppression led to the inhibition of EAC.

To compare the effects of timing of FK506 administration at the same amount, we injected 200 µg of FK506 either during early induction phase (from day 0 to day 6) or late induction phase (from day 7 to day 13). At this dose, there was no remarkable difference in inhibitory effects on EAC between the two phases (Fig. 2). Although cellular proliferative responses were inhibited in both groups, (Fig. 3a, b) antibody production was not suppressed in the group treated during late induction phase (Fig. 3b, c). These data further supported our previous report that cellular immunity, rather than humoral immunity, is involved in the development of EAC [8]. In addition, the data could be interpreted as indicating that once B cells were primed with OVA, their function could not be disturbed by FK506, while function of T cells could be inhibited by FK506 even after being primed.

Although the number of CD4-positive T cells in the group treated with 200 µg of FK506 is lower than in the control group, there were definitely still CD4-positive T cells in the former group (Table 2). Previous reports demonstrated that the frequency of soluble antigen-specific cells in immunized animals was in general lower than 1% [4], and therefore, a 20% decrease in CD4-positive T cells in the FK506-treated group may also depend on a decrease in the cell population not specific to OVA. Taking Table 1 into consideration, it could be regarded that not only OVA-specific but also OVA-unrelated CD4-positive T cells were disturbed by FK506 treatment. Therefore, the suppressive effects on EAC of the systemic administration of FK506 could be partially attributed to a decrease in OVA-unrelated CD4-positive lymph node cells as well as OVA-specific cells.

It is also of note that CD8-positive cells were not affected by FK506, although we did not test the function of these cells. As FK506 specifically blocks the signaling pathway initiated by T cell receptor [2, 18, 19], CD4-positive macrophages should not be affected. From these data, as previously reported [12], FK506 specifically suppressed CD4-positive T cells without affecting other phenotypes of cells such as CD8-positive T cells and macrophages.

Although the data on FK506 treatment in actively immunized rats indicated efficient suppression of EAC by FK506, it could be considered that this inhibition might depend on the suppression of the maturation of CD4-pos-

itive T cells during the induction stage. To test the effectiveness of FK506 during the effector phase, we injected FK506 into rats that had received OVA-primed lymph node cells. Even though we did not clarify whether FK506 inhibited the functions of donor cells, recipient cells or both, FK506 treatment during the effector phase inhibited the development of EAC (Fig. 4a) as well as cellular proliferative responses against OVA (Fig. 5). Including the data of actively immunized rats, we concluded that systemic administration of FK506 is effective for inhibition of EAC in both the induction and the effector phases.

Because EAC is a T cell-mediated disease [8] (unpublished results), and FK506 should be easily available to the conjunctiva in the form eye drops, there is a good possibility that topical administration of FK506 might be effective for inhibition of disease. Some inhibition, albeit not statistically significant, was observed for treatment with topical FK506 (Fig. 4b). Although we used FK506 at one particular concentration (0.3% weight per volume), this concentration was demonstrated to be effective for suppression of endotoxin-induced uveitis [15]. With respect to cyclosporin A, a 0.4% concentration was effective to suppress the development of mast cell-mediated conjunctivitis [22]. However, the induction mechanism of EAC is different, and therefore this concentration of topical FK506 may not be high enough to inhibit EAC.

Dose-dependency of FK506 on inhibition (Fig. 1a, Table 1) indicated that the amount administered in vivo is important for the effectiveness of FK506. The route of administration is also important, because topical application by eye drops must affect infiltrated cells more directly. Concerning the adoptive transfer system in this study, the total amount of FK506 given by systemic administration was 1 mg, while that by topical was approximately 4.2 µg. Therefore, a possible explanation for differences in effectiveness of FK506 between systemic and topical administration is that systemic administration may lead to a higher and continuous FK506 concentration in blood and also to a higher concentration of FK506 in the conjunctiva.

The data presented here demonstrate, for the first time, the effective inhibition by FK506 of the development of experimental allergic conjunctivitis induced by exogenous antigens. From these data, FK506, especially by systemic administration, may be a candidate for the therapy of certain types of cell-mediated conjunctivitis in humans. In addition, topical administration of FK506, in combination with other drugs such as topical steroids, may be effective in the treatment of allergic conjunctivitis.

Acknowledgement We would like to thank Ms. Natsuko Naruoka for her technical help in preparation of histological slides.

References

1. Abu el-Asrar AM, Van den Oord JJ, Geboes K, Missotten L, Emarah MH, Desmet V (1989) Immunopathological study of vernal keratoconjunctivitis. *Graefes Arch Clin Exp Ophthalmol* 27:374-379
2. Banerji SS, Parsons JN, Tocci MJ (1991) The immunosuppressant FK506 specifically inhibits mitogen-induced activation of the interleukin-2 promoter and the isolated enhancer elements NF IL-2A and NF-AT1. *Mol Cell Biol* 11:4074-4087
3. Colby K, Dohlman C (1996) Vernal keratoconjunctivitis. *Int Ophthalmol Clin* 36:15-20
4. Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM, Strober W (1992) Current protocols in immunology, 1st edn. Greene Wiley-Interscience, New York
5. Doherty MJ, Easty DL (1989) Inflammatory and immunological cell profiles in a rat model of conjunctival immediate hypersensitivity. *Clin Exp Allergy* 19:449-455
6. Donshik PC (1988) Allergic conjunctivitis. *Int Ophthalmol Clin* 28:294-302
7. Fujino Y, Mochizuki M, Chan CC, Raber J, Kotake S, Gery I (1991) FK506 treatment of S-antigen induced uveitis in primates. *Curr Eye Res* 10:679-690
8. Fukushima A, Yoshida H, Iwamoto H, Yoshida O, Ueno H (1997) The role of cellular immunity both in the induction and effector phases of experimental allergic blepharoconjunctivitis (EAC) in rats. *Exp Eye Res* 65:631-637
9. Hikita N, Chan CC, Whitcup SM, et al. (1995) Effects of topical FK506 on endotoxin-induced uveitis (EIU) in the Lewis rat. *Curr Eye Res* 14:209-214
10. Hikita N, Lopez JS, Chan CC, Mochizuki M, Nussenblatt RB, de Smet MD (1997) Use of topical FK506 in a corneal graft rejection model in Lewis rats. *Invest Ophthalmol Vis Sci* 38:901-909
11. Hingorani M, Lightman S (1995) Therapeutic options in ocular allergic disease. *Drugs* 50:208-221
12. Kawashima H, Fujino Y, Mochizuki M (1988) Effect of a new immunosuppressive agent, FK506, on experimental autoimmune uveoretinitis in rats. *Invest Ophthalmol Vis Sci* 29:1265-1271
13. Lauerma AI, Maibach HI, Granlund H, Erkkö P, Kartamaa M, Stubb S (1992) Inhibition of contact allergy reactions by topical FK506. *Lancet* 340:556
14. Lauerma AI, Stein BD, Homey B, Lee CH, Bloom E, Maibach HI (1994) Topical FK506: suppression of allergic and irritant contact dermatitis in the guinea pig. *Arch Dermatol Res* 286:337-340
15. Metz DP, Bacon AS, Holgate S, Lightman SL (1996) Phenotypic characterization of T cells infiltrating the conjunctiva in chronic allergic eye disease. *J Allergy Clin Immunol* 98:686-696
16. Mochizuki M, Kuwabara T, McAllister C, Nussenblatt RB, Gery I (1985) Adoptive transfer of experimental autoimmune uveoretinitis in rats. Immunopathogenic mechanisms and histologic features. *Invest Ophthalmol Vis Sci* 26:1-9
17. Mochizuki M, Masuda K, Sakane T, Ito K, Kogure M, Sugino N, et al. (1993) A clinical trial of FK506 in refractory uveitis. *Am J Ophthalmol* 115:763-769
18. O'Keefe SJ, Tamura J, Kincaid RL, Tocci MJ, O'Neill EA (1992) FK506 and CsA-sensitive activation of the interleukin-2 promoter by calcineurin. *Nature* 357:692-694
19. Sawada S, Suzuki G, Kawase Y, Takaku F (1987) Novel immunosuppressive agent, FK506. In vitro effects on the cloned T cell activation. *J Immunol* 139:1797-1803
20. Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramman R, Jain A (1989) FK506 for liver, kidney, and pancreas transplantation. *Lancet* 2:1000-1004
21. Todo S, Demetris A, Ueda Y, Imv-entanza O, Cadoff E, Zeevi A, et al. (1989) Renal transplantation in baboons under FK506. *Surgery* 106:444-450
22. Whitcup SM, Chan CC, Luyo DA, Bo P, Li Q (1996) Topical cyclosporine inhibits mast cell-mediated conjunctivitis. *Invest Ophthalmol Vis Sci* 37:2686-2693