Treatement of Experimental Autoimmune Uveoretinitis with Intravitreal Injection of Tacrolimus (FK506) Encapsulated in Liposomes

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PURPOSE. To evaluate the effects of intravitreal injection of liposomes encapsulating tacrolimus (FK506) on experimental autoimmune uveoretinitis (EAU) in Lewis rats.

METHODS. Liposomes containing tacrolimus were prepared by reverse-phase evaporation vesicles. EAU was induced in Lewis rats by subcutaneous injection of interphotoreceptor retinoid-binding protein R16 peptide emulsified in adjuvant. Ten days later, rats were intravitreally injected with saline, tacrolimus, tacrolimus-loaded liposomes, or unloaded liposomes. Clinical signs of inflammation and ocular histologic sections were observed and graded. Retinal function was evaluated by electroretinography (ERG). Tacrolimus concentration was determined in the vitreous body and serum by ELISA. Ocular biodistribution of rhodamine-conjugated liposomes containing tacrolimus (tacrolimus-Rh-lip) was analyzed with a laser scanning confocal microscope. To evaluate the systemic effect of intravitreally injected tacrolimus, delayed-type hypersensitivity (DTH) and lymphocyte proliferation assay (LPA) responses were detected.

RESULTS. Treatment of EAU with intravitreal injection of liposomal tacrolimus significantly reduced intraocular inflammation and markedly inhibited the development of EAU, as determined in clinical and histopathologic analyses. No toxic effects could be detected as evaluated by ERG. The concentration of tacrolimus in ocular fluids remained for as long as 14 days after liposomal injection of tacrolimus. Confocal microscopy showed a transretinal distribution of the liposomal particles. DTH and LPA responses were not impaired in liposomal tacrolimus-treated rats.

CONCLUSIONS. Intravitreal injection of liposomal tacrolimus was highly effective in suppressing the process of EAU without any side effects on retinal function or systemic cellular immunity. This treatment may represent a new option for the management of intraocular inflammation. (Invest Ophthal Vis Sci. 2010;51:3575–3582) DOI:10.1167/iovs.09-4373

Materials and Methods

Animals

Eight- to 10-week-old inbred female Lewis rats, each weighing 150 to 180 g, were used in this study. All rats were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Induction and Assessment of EAU

EAU was induced by injection of 30 μg interphotoreceptor retinoid-binding protein (IRBP) R16 peptide (a uveitogenic peptide) emulsified in complete Freund’s adjuvant (CFA; Sigma, St. Louis, MO) containing 2.5 mg/ml killed Mycobacterium tuberculosis H37Ra into the left hind footpads of the Lewis rats. Each day for 25 days after IRBP R16
immunization, eyes were examined independently by two masked observers to assess the clinical signs of uveitis using a slit-lamp biomicroscope. Clinical signs were scored as follows: 0, normal; 1, minimal signs of inflammation with occasional cells in the anterior chamber; 2, presence of mild exudate in the anterior chamber; 5, moderate exudate in the anterior chamber or moderate exudate in the posterior chamber (posterior chamber hypopyon); 4, large exudate within the anterior chamber or massive posterior chamber hypopyon; 5, gross orbital edema and exophthalmos in addition to large exudate within the anterior chamber or massive posterior chamber hypopyon.14

Preparation and Characterization of Tacrolimus-Lip

Liposomes containing tacrolimus were prepared by reverse-phase evaporation vesicles, as previously described.15 Briefly, a mixture of phospholipids and cholesterol, in a ratio of 10:1 (wt/wt), was dissolved in 12 ml chloroform. The organic solvents were evaporated under reduced pressure with a rotary evaporator (R-201; Shanghai Science and Education Equipment Co., Ltd., Shanghai, China) at 40°C such that a thin dry film of the components was formed. The film was redissolved in 12 ml ether, and a solution containing 20 mg tacrolimus (Prograf; Fujisawa, Osaka, Japan) in 4 ml acetone together with 6 ml PBS (pH 7.4) was added. The mixture was sonicated (600 W), and the resultant opalescent dispersion was rotary evaporated to disrupt the gel formed immediately. After the addition of 10 ml PBS (pH 7.4), rotary evaporation was continued for an additional 15 minutes to ensure the removal of residual diethyl ether. The suspension was stored at 4°C. Rhodamine-conjugated liposomes (tacrolimus-Rh-lip) were also prepared as mentioned except that rhodamine B (0.3%) was added in PBS. For sterility, all these steps were performed under aseptic conditions. All glassware was sterilized by autoclaving, PBS was added in PBS. For sterility, all these steps were performed under aseptic conditions. All glassware was sterilized by autoclaving, PBS was passed through a 0.22-μm membrane filter, and the entire procedure was carried out in a laminar flow hood. The formation and dispersion of liposomes were characterized by scanning electron microscopy (SIRION 200; FEI Company, Hillsboro, OR). Vesicle sizes of the liposomes were determined with a high-performance particle sizer (λ = 632.8 nm; Malvern Instruments Ltd., Worcestershire, UK). Encapsulation efficiency is defined as follows:16

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\text{Tacrolimus entrapment efficiency (\%) = } \frac{\text{amount of tacrolimus entrapped/total amount of tacrolimus}}{100}
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Intravitreal Injection Protocols

Lewis rats were anesthetized by intraperitoneal injection of 0.15 ml pentobarbital. Pupils were dilated by instillation of 1 drop of tropicamide 5%. One drop of tetracaine 1% was administered for local anesthesia. Intravitreal injections (5 μL) were performed 10 days after the induction of EAU. Rats were randomly assigned to 1 of 4 groups and were treated with PBS, tacrolimus, tacrolimus-loaded liposomes, or unloaded liposomes in both eyes using sterile syringes fitted with a 30-gauge needle, as previously described.17 Four rats chosen randomly from each group were monitored and examined with a slit-lamp biomicroscopy for 30 days, whereas the other rats in these groups were killed at different times after injection for further study, as described below. Four normal rats were injected intravitreally with tacrolimus-lip to determine the safety of the treatment. To quantify the concentration of tacrolimus in the injected eyes and peripheral blood, EAU rats that received tacrolimus or tacrolimus-lip were killed at 6 hours, 24 hours, 7 days, and 14 days after intravitreal injection (n = 4 per group). Vitreous bodies of both eyes of each animal were collected; tacrolimus concentration was then determined by a specific ELISA kit (DiaSorin, Stillwater, MN), with a lower detection limit of 0.5 ng/mL. Concentrations of tacrolimus in the sera of rats injected with tacrolimus-lip and free-tacrolimus were also measured to determine possible systemic adverse effects.

Histopathologic Assay of EAU after Intravitreal Injection

Histopathologic examinations of the enucleated eyeballs were performed on days 14 and 17 after immunization (n = 4 per group). Eyes of the rats killed in each group were enucleated and fixed in 10% formaldehyde. Sections 5-μm thick were stained with hematoxylin and eosin and were examined with a light microscope. Histologic grading was as follows: 0, no inflammatory cell infiltration and no destruction of the retina; 1, minimal cell infiltration in the retina and choroid but no destruction; 2, partial and mild destruction of the outer retina; 3, moderate destruction of the outer retina; 4, extensive and severe destruction of the outer retina and partial destruction of the inner retina; 5, complete destruction of the entire retina.18

Confocal Microscopy Analysis of the Localization of Tacrolimus-Rh-Lip

Seventeen EAU rats were used for the ocular biodistribution study of rhodamine-conjugated liposomes containing tacrolimus (tacrolimus-Rh-lip). Intravitreal injection of tacrolimus-Rh-lip (5 μL) was performed 10 days after the induction of EAU. Control rats received intravitreal injections of saline (n = 2). At 6 hours, 24 hours, 7 days, 14 days, and 21 days (n = 3 per time point and per group) after intravitreal injection, the eyes of the killed rats in each group were enucleated, and the eyecups were fixed in 4% paraformaldehyde solution for 2 hours after removal of the cornea and the lens. The eyecups were cryoprotected in graded sucrose solutions (20%-30% in PBS) at 4°C, embedded and frozen in optimal cutting temperature compound (Tissue-Tek; Ted Pella, Inc., Redding, CA), and stored at −80°C. Ten-micrometer sections were cut using a cryostat (Bright Instruments Ltd., Huntingdon, UK). The sections were examined under a confocal laser microscope (TCS SP2; Leica Microsystems, Wetzlar, Germany) to characterize the biodistribution of tacrolimus-Rh-lip.

Electroretinography

Retinal function was evaluated by scotopic electroretinography (ERG; UTAS-E3000 Visual Electrodiagnose System; LKC Technologies, Gaithersburg, MD) 5 days after intravitreal injection of tacrolimus-lip in normal rats. Normal rats without intravitreal injection were evaluated simultaneously. Rats were dark adapted for 15 minutes after systemic anesthesia. One drop of tetracaine 1% was administered for local anesthesia. Pupils were dilated with tropicamide 5%. Topical methylcellulose 1% was used as the conducting medium. A golden-ring electrode was placed on the cornea. Reference and ground electrodes were attached to the forehead and the trunk of each animal. Ten responses to a 2500 cd/m² white light flash (10 μs, 0.1 Hz) from a Ganzfeld integrating sphere were amplified and averaged (1902 Signal Conditioner/I401 Laboratory Interface; CED, Cambridge, UK). The b-wave amplitude was measured from the trough of the a-wave to the peak of the b-wave, and the a-wave was measured as the difference in amplitude between the recording at 5 ms and the trough of the negative deflection.19

Assay for Delayed-type Hypersensitivity

Delayed-type hypersensitivity (DTH) responses to IRBP R16 peptide were determined by measuring the thickness of the swelling ears. On day 13 after IRBP R16 immunization, 20 μg IRBP R16 peptide in 20 μL PBS per rat was injected into the right pinnae. PBS was injected into the left pinnae as a control. Ear thickness was measured with a micrometer 24 hours after R16 peptide challenge. DTH-dependent ear swelling was calculated according to the following formula: specific ear swelling = [(24-hour measurement of right ear − 0-hour measurement of right ear) − (24-hour measurement of left ear − 0-hour measurement of left ear)].18

Lymphocyte Proliferation Assay

Draining (inguinal) lymph nodes were collected, and a single-cell suspension was prepared. Lymphocyte proliferation assay (LPA) was

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*Source: IOVS, July 2010, Vol. 51, No. 7*
Therapeutic Effect of Intravitreal Injection of Tacrolimus and Tacrolimus-Lip on Ocular Inflammatory Response

Intravitreal injection of PBS, tacrolimus, tacrolimus-loaded liposomes, or unloaded liposomes was performed on day 10 after immunization. The effect of intravitreal injection on ocular inflammation in the anterior segment was determined by the clinical score. In EAU rats, intravitreal injection of unloaded Lip had no therapeutic effect on ocular inflammation compared with that in rats receiving saline intravitreally, as shown in Figure 2 (mean clinical scores on day 4 after injection were 3.20 ± 0.54 and 3.60 ± 0.45, respectively). By contrast, intravitreal injection of tacrolimus-lip and tacrolimus significantly reduced intraocular inflammation as evaluated by slit-lamp biomicroscopy (mean clinical scores on day 4 after injection were 1.00 ± 0.70 and 0.40 ± 0.55, respectively). Furthermore, the inflammatory response in the anterior segment of tacrolimus-lip–treated eyes was totally diminished 7 days after intravitreal injection, whereas tacrolimus-treated eyes still showed mild signs of EAU (Fig. 2). Consistent with the clinical signs, histologic analysis of eyes taken at day 14 after immunization (4 days after intravitreal injection) revealed that the rats received PBS, and unloaded Lip showed marked features of EAU characterized by severe retinal destruction associated with infiltration of numerous inflammatory cells (Figs. 3A, 3C). Histologic grades were 4.5 ± 0.58 and 4.25 ± 0.5, respectively. By contrast, intravitreal injection of tacrolimus and tacrolimus-lip markedly reduced the histologic grading of uveitis, and the architecture of retinal layers was preserved (Figs. 3E, 3G). Histologic grades were 2.75 ± 0.5 and 2.25 ± 0.5 (P < 0.05), respectively.

Seven days after intravitreal injection, numerous inflammatory cells were also seen throughout the retinal layers of rats treated with PBS and unloaded liposomes (Figs. 3B, 3D). Inflammatory cell infiltration was significantly reduced with mild destruction of the outer retina in the tacrolimus-lip–treated eyes, whereas there was minimal or no cell infiltration without destruction of the retina in the tacrolimus-lip–treated eyes. (Figs. 3F, 3H). Histologic grades were 1.75 ± 0.75 and 0.75 ± 0.5 (P < 0.05), respectively. No inflammatory sign was detected in any eyes of normal rats given intravitreal injection of tacrolimus-lip.

Biodistribution of Rhodamine-Conjugated Liposomes in the Retinas of EAU Rats

The intraocular biodistribution of tacrolimus-Rh-lip at various time points after intravitreal injection is shown in Figure 4. Six hours after intravitreal injection, fluorescent liposomes (red) containing tacrolimus were detected primarily along the retinal inner limiting membrane of the retina and in the vitreous body (Fig. 4A). At 24 hours, large numbers of liposomes accumulated from internal limiting membranes to outer nuclear layers.
(Fig. 4B). At days 7 and 14, the numbers of liposomes decreased in the retina, whereas the distribution of liposomes was internalized (Figs. 4C, 4D). At 21 days, there were still scattered liposomes within the retina (Fig. 4E). Retinas of control rats showed no fluorescence (figure not shown).

**Tacrolimus Concentration in Vitreous Body and Serum**

Tacrolimus concentration in the vitreous body decreased as a function of time in rats receiving intravitreally administered tacrolimus and tacrolimus-lip (Fig. 5). Tacrolimus concentrations were higher 6 hours after tacrolimus injection than after tacrolimus-lip injection ($710 \pm 205$ ng/mL vs. $602 \pm 173$ ng/mL; $P < 0.05$). However, at 24 hours, tacrolimus concentration was twice higher after tacrolimus-lip injection than after tacrolimus injection ($150 \pm 43$ ng/mL vs. $72 \pm 19$ ng/mL; $P < 0.05$). Seven days after injection, the concentration of tacrolimus in the vitreous body after injection was under the detection limit of the kit (0.5 ng/mL), whereas the concentration of tacrolimus-lip injection was $75 \pm 16$ ng/mL and remained higher at 14 days after injection ($50 \pm 15$ ng/mL). Concentrations of tacrolimus in the sera of rats receiving intravitreal injections of tacrolimus and tacrolimus-lip

**FIGURE 3.** Effect of intravitreal injection on histopathologic changes (on days 4 and 7 after injection) in the posterior segment of eyes with EAU (hematoxylin and eosin staining; original magnification, $\times 200$). Representative results are shown of histopathologic features of the retina 4 days after intravitreal injection of PBS (A), unloaded liposomes (C), tacrolimus (E), and tacrolimus-loaded liposomes (G) and 7 days after intravitreal injection of PBS (B), unloaded liposomes (D), tacrolimus (F), and tacrolimus-loaded liposomes (H).
were under the detection limit of the kit throughout the entire course of observation.

**Toxicity Evaluation of Intravitreal Administration of Tacrolimus-Lip**

No inflammatory sign in the anterior chamber was detected in any eye in normal rats receiving intravitreal injections of tacrolimus-lip. Cataract was not observed during the observation period. Mild vitreous opacity was observed in the tacrolimus-lip–treated group. Histopathologic analysis did not show any evidence of drug-released toxic effect in the tacrolimus-lip injection group. In addition, ERG was performed to detect the effects of intravitreal injection on the function of the retina. Tacrolimus-lip was injected into the vitreous cavity of normal rats 5 days before ERG. Based on ERG findings, no changes in waveform or amplitude for a-waves or b-waves were observed in tacrolimus-lip–injected eyes compared with untreated normal control eyes (Fig. 6).

**Delayed-type Hypersensitivity Responses after Intravitreal Injection**

To evaluate the extent of the impact of intravitreal injection on the immune system, we measured DTH in vivo. DTH responses

![Figure 4](image4.png)

**Figure 4.** Ocular biodistribution of rhodamine-conjugated liposomes containing tacrolimus-Rh-lip in EAU rats. At 6 hours after intravitreal injection of tacrolimus-Rh-lip, liposomes (red) are observed in the vitreous body and along the internal limiting membrane of the retina (A). At 24 hours, liposomes accumulate in substantial numbers from the internal limiting membrane to the outer nuclear layer (B). At 7 and 14 days, liposomes are internalized throughout the retina but with decreased numbers (C, D). At 21 days, liposomes are still detectable within the retina (E).

![Figure 5](image5.png)

**Figure 5.** Tacrolimus concentrations in vitreous body after intravitreal injection of tacrolimus and tacrolimus-lip in EAU rats. Values represent mean ± SD; n = 4 for each time point.

![Figure 6](image6.png)

**Figure 6.** Scotopic electroretinogram recorded in rats. (A) Actual ERG traces of normal rat. (B) Actual ERG traces of eyes at 4 days after intravitreal injection of tacrolimus-lip.
were typically manifested by the swelling of ear pinnae. There were no significant differences in DTH responses to IRBP R16 peptide among the rats treated with intravitreal injections of saline, tacrolimus, tacrolimus-lip, or unloaded liposomes (Fig. 7).

Effect of Intravitreal Injection on IRBP R16-Specific Lymphocyte Proliferation Assay

To observe the effects of intravitreal injection on the function of IRBP R16 specific T cells, we evaluated antigen-specific lymphocyte activation at the peak of EAU. Fourteen days after immunization with IRBP R16 peptide, antigen-specific proliferation of cells from draining lymph nodes was observed in the presence or absence of the peptide. Intravitreal injections of tacrolimus and tacrolimus-lip did not change the proliferation of uveitogenic T cells during EAU (Fig. 8). These data suggest that intravitreal injection of tacrolimus and tacrolimus-lip do not suppress ocular inflammation through the regulation of antigen-specific lymphocyte activation.

DISCUSSION

Liposomes are membrane-like vesicles consisting of one or more concentric phospholipid bilayers of alternating aqueous and lipophilic compartments, making them potential carriers for lipophilic drugs. Liposomes have a greater affinity for the retina, providing high local availability. In addition, liposomes are degradable to achieve the controlled release of drugs and to enhance the efficacy of topical administration to avoid repeated injections. These properties make them good candidates for intraocular delivery. In the present study, we prepared the tacrolimus-lip using reverse-phase evaporation vesicles and observed its safety and efficacy during injection into the vitreous cavity. We found that the treatment was well tolerated and very efficient in ameliorating the severity of EAU for a prolonged time after a single administration.

The intraocular tolerance of tacrolimus-loaded liposomes was tested in normal rats. No inflammation was observed in the injected eyes during evaluation by slit-lamp biomicroscopy. Mild vitreous opacity observed in the tacrolimus-lip–treated group after injection was considered to be caused by the dispersion of liposomes in the vitreous cavity. We were able to minimize the quantity of liposomes needed in this treatment strategy by increasing the encapsulation efficiency of liposomes and found the concentration for optimal therapy effect and the lowest vitreous opacity. Histopathologic examination results of the retina were normal compared with those of untreated control. In the evaluation of retinal function using ERG, there was no evidence of toxic effects for a-wave or b-wave in tacrolimus-lip injection groups. We thus confirmed the safety of tacrolimus-lip when administered intravitreally. Further studies will be necessary to adequately predict safety after a long period. Indeed, the intravitreal injection of either tacrolimus or unloaded liposomes was histologic and functional safety without side effects as reported previously. Liposomal encapsulation of tacrolimus leads to a reduced amount of free tacrolimus in direct contact with the ocular tissues so as to reduce local toxicity. In addition, this treatment regimen did not increase the serum concentration of tacrolimus at all time points. Pharmacologic effects of the drug were manifested only through local actions in the eye, not by way of a systemic route; hence, systemic side effects were minimized. Thus, the use of relatively high concentrations of liposomes with large amounts of tacrolimus may be feasible without leading to potential adverse effects.

In this study, we demonstrated that intravitreal injection of tacrolimus-lip or tacrolimus was efficient in delaying the onset of EAU and ameliorating the severity of ocular inflammation. The substantially reduced EAU clinical scores of tacrolimus-lip–treated and tacrolimus-treated rats correlated histologically with the mitigated retinal damage and immune cell infiltration. In contrast, the control rats that received PBS and unloaded-lip showed severe retinal destruction and numerous inflammatory cells in the anterior chamber, vitreous, and retina. These results are consistent with previous findings that intravitreal injection of tacrolimus or sustained release of tacrolimus can be effective for experimental uveitis in a rabbit model. EAU induced in Lewis rats by IRBP or IRBP R16 peptide has a markedly shorter course than does uveitis in human patients. EAU clinical changes disappeared spontaneously within 14 days after immunization, with no residual apparent changes in the anterior segment. Accordingly, we observed the clinical efficacy of tacrolimus compared with liposomal tacrolimus only up to 14 days after injection. Long-term study is necessary to determine treatment efficacy in humans with chronic uveitis.

Tacrolimus has been formulated into liposomes to improve its protection against degradation. After intravitreal injection of tacrolimus-Rh-lip in EAU rats, Rh-labeled liposomes and cells bearing liposomes were located mainly in the vitreous and inner layers of the retina. The liposomes dispersed in the retina thereafter, and the localization of particles was observed in vitreous body and all layers of the retina 24 hours after intravitreal injection and was still visible after 3 weeks, whereas the amount of liposomes decreased with time. Histopathologically, the injection protocol greatly reduced inflammatory cells and untreated control. In the evaluation of retinal function using ERG, there was no evidence of toxic effects for a-wave or b-wave in tacrolimus-lip injection groups. We thus confirmed the safety of tacrolimus-lip when administered intravitreally. Further studies will be necessary to adequately predict safety after a long period. Indeed, the intravitreal injection of either tacrolimus or unloaded liposomes was histologic and functional safety without side effects as reported previously. Liposomal encapsulation of tacrolimus leads to a reduced amount of free tacrolimus in direct contact with the ocular tissues so as to reduce local toxicity. In addition, this treatment regimen did not increase the serum concentration of tacrolimus at all time points. Pharmacologic effects of the drug were manifested only through local actions in the eye, not by way of a systemic route; hence, systemic side effects were minimized. Thus, the use of relatively high concentrations of liposomes with large amounts of tacrolimus may be feasible without leading to potential adverse effects.

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preserved the architecture not only of the inner layers but also of the outer layers of the retina, whereas free-tacrolimus treatment appeared to be insufficient at a later stage of inflammation. The encapsulation of tacrolimus in liposomes increased its therapeutic effects for a prolonged time compared with free-tacrolimus, as previously described for several drugs.28–30 Intravitreally administered tacrolimus-Rh-lip penetrated the inner layers to achieve transretinal targeting, and the formulation acted as a reservoir in ocular tissues and released tacrolimus gradually at the site of inflammation. Targeting efficacy toward inflamed tissue has been found to be dependent on the particle diameter of the carrier system.31 Small microspheres have been proved to have pharmacologic efficacy in this context.32 Drug-loaded liposomes offer several advantages over other particles, such as a higher adhesive capacity and enhanced drug penetration into the inflamed tissues, suggesting that liposomes can be effective vehicles for local treatment in ocular disorders, including uveitis.33

Although the minimum intravitreous concentration necessary for the treatment of uveitis is unknown, we found in our study that liposomes were able to deliver drug to the vitreous body at concentrations higher than 50 ng/mL for 14 days and that these tacrolimus concentrations were effective in controlling the intraocular inflammation of EAU. Previous studies34 demonstrated that drugs formulated into liposomes could provide long-lasting protection against ocular disease for 4 to 6 weeks after a single intravitreal injection. In addition, some approaches were used to achieve prolonged retention of drug-bearing liposomes (up to 4 months) within ocular tissues.35,36 Long-term study is also needed to determine the duration of efficacy of tacrolimus-loaded liposomes.

Intravitreal administration of tacrolimus-lip has the important advantage of greatly augmenting the drug concentration at the target site while reducing the total drug burden. Tacrolimus-lip might approach the retina through the breached blood-retinal barrier secondary to inflammation and thereby suppress tissue damage.37 Moreover, cells incorporating tacrolimus-lip could participate in the retention or slow release of tacrolimus in the eye, possibly changing the intraocular immune environment over a long period.24

To evaluate the possible effect of intraocular injection of tacrolimus-lip on the systemic immune system, we measured DTH in vivo and antigen-specific lymphocyte proliferation activation in vitro. We found that neither DTH nor LPA was suppressed in liposomal tacrolimus-treated rats, indicating that intravitreal injection of tacrolimus-lip did not ameliorate EAU though the generation of antigen-specific inhibition. Moreover, because serum levels of tacrolimus were undetectable at all time points, we concluded that the effect of therapy was manifested only through local actions without affecting the systemic responses.

In conclusion, our results indicate that intravitreal injection of tacrolimus-lip can directly target ocular inflammation sites and suppress autoimmune uveoretinitis effectively. Liposomes allowed tacrolimus to release gradually in a stable manner for 14 days, and tacrolimus-lip injected into the vitreous cavity as a single administration can suppress the inflammation of ocular tissues for a long period without potential adverse effects. Thus, intravitreal injection of tacrolimus encapsulated in liposomes could be an attractive strategy for local treatment of patients with chronic uveitis who need sustained release of the drug.

References


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