Ocular Toxicity of Intravitreal Tacrolimus

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BACKGROUND AND OBJECTIVE: To investigate the ocular toxicity of intravitreally administered tacrolimus, a drug with potent immunosuppressive activity.

METHODS: To evaluate toxicity, tacrolimus was injected into the midvitreous cavity of 20 eyes of New Zealand pigmented rabbits at concentrations of 10, 50, 100, 250, 500, and 1000 µg. Control eyes received balanced salt solution. Eyes receiving 1000 µg were given injections of 0.2 mL solution; all others, including controls, received 0.1 mL. Rabbits were examined before the injections by slit-lamp biomicroscopy, indirect ophthalmoscopy, and an electroretinography test (ERG) was performed. The animals were followed up to 14 days postinjection by clinical examination and ERG. The animals were killed and the eyes were enucleated and processed for light microscopy.

RESULTS: No evidence of a retinal toxic reaction was seen in the eyes receiving 10 or 50 µg of tacrolimus. One out of 4 eyes that received 100 µg of the drug developed a vitreous reaction. All eyes treated with 250 µg or more developed vitreous reaction. One eye injected with 1000 µg of the drug developed occlusion of the temporal retinal vessels. Electrotinography showed decreasing b-wave amplitude with both dark- and light-adapted stimulus in the 500 and 1000 µg groups, and it was normal in the other groups. Histopathologic sections showed mild disorganization of the retina only at the 500 and 1000 µg dosage.

CONCLUSIONS: Doses of 10 and 50 µg of tacrolimus are nontoxic to rabbit eyes. Only transient vitreous opacities were observed in the groups that received 100 and 250 µg. Intravitreal doses of 500 and 1000 µg of tacrolimus proved to be toxic to the retina.

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INTRODUCTION

Tacrolimus, previously known as FK 506, is a neutral macrolide compound isolated from the fermentation broth of a strain of soil fungus, Streptomyces tsukubaensis.1 This drug has been demonstrated to have potent immunosuppressive activity with immunopharmacological features similar to cyclosporine A. Although the action of tacrolimus resembles that of cyclosporine A, it is generally found to show comparable effectiveness both in vivo and in vitro at concentrations two or three orders of magnitude lower.2 Tacrolimus acts primarily on CD4+ T helper lymphocytes by inhibiting the production of lymphokines, especially interleukin-2. This drug also inhibits the release of proinflammatory and vasoactive mediators from mast cells and basophils.3,4

In experimental studies in animals, tacrolimus prevented graft rejection and prolonged graft survival in kidney, liver, and heart transplantations.5-7 The immunosuppressive properties of tacrolimus have been confirmed in humans who underwent kidney, liver, and heart transplantation.8-10

Tacrolimus was used in the treatment of experimental autoimmune uveoretinitis in animals, and was shown to suppress the cellular and humoral immune
responses to some extent. In humans, tacrolimus used systemically to treat refractory uveitis has been effective in a dosage-dependent manner. However, this therapy induced a variety of adverse side effects.

Despite its systemic use in ophthalmology, there is no report of its intravitreal use. This study was undertaken to investigate the ocular toxicity of intravitreally administered tacrolimus.

**MATERIALS AND METHODS**

**Drug**

The drug tacrolimus used in this study was commercially obtained from Prograf, Fujisawa Healthcare, Inc., Deerfield, IL and stored at 24°C. The undiluted preparation had a tacrolimus concentration of 5 mg/mL. The drug was diluted in sterile balanced salt solution to concentrations of 10, 50, 100, 250, 500, and 1000 µg.

**Intravenous Injection**

Twenty eyes of New Zealand pigmented rabbits, weighing 2 to 3 kg were used in this study. All animal care and euthanasia were in strict conformity with the Association for Research in Vision and Ophthalmology guidelines for use of animals in research. Before intravitreal injection, the eyes were examined with slit-lamp biomicroscopy, indirect ophthalmoscopy, and an electroretinography test (ERG) was performed.

The rabbits were anesthetized with an intramuscular injection of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (5 mg/kg). The pupils were dilated with 2.5% phenylephrine and 0.5% tropicamide. Topical anesthesia was achieved with 0.5% proparacaine hydrochloride. Before the intravitreal injection, the eyes were sterilized with 5% povidone-iodine topically. A paracentesis was performed with a 30-gauge needle to lower the intraocular pressure. The different doses of tacrolimus or balanced salt solution were then injected into the midvitreous cavity, 3 mm posterior to the limbus superonasally, using a 30-gauge needle on a 1-mL tuberculin syringe with the beveled side of the needle facing anteriorly. The precise location of the needle could be viewed through the dilated pupil. No apparent leakage of fluid from the injection site occurred during any of the injections. Immediately after the injection, biomicroscopy and indirect ophthalmoscopy were again performed to rule out injection complications. These examinations were repeated in all eyes on days 1 and 2 after injection and every other day thereafter for 14 days.

The eyes were divided in seven groups: group 1, 3 eyes given 10 µg of the drug in 0.1 mL of solution; group 2, 3 eyes given 50 µg of the drug in 0.1 mL of solution; group 3, 4 eyes given 100 µg of the drug in 0.1 mL of solution; group 4, 2 eyes given 250 µg of the drug in 0.1 mL of solution; group 5, 3 eyes given 500 µg of the drug in 0.1 mL of solution; group 6, 3 eyes given 1000 µg of the drug in 0.2 mL, and group 7, 2 eyes given 0.1 mL of diluent as control. The contralateral eyes were left untouched.

**Electroretinography Test**

The ERG tests were performed both before and 7 and 14 days after intravitreal injection. These tests were done using the UTAS-E 2000 system (LKC Technologies, Gaithersburg, MD).

The animals were anesthetized and their pupils were dilated as previously described. The rabbits were dark-adapted for 20 minutes before tests. Positive unipolar contact lenses were placed on both corneas with gonioscopic solution. The negative electrode was put into the subcutaneous space of the forehead and the ground electrode was clipped to the earlobe. Three sweeps were averaged for each step. Steps 1 and 2 were a scotopic white 24 dB single flash and a scotopic white 0 dB single flash, respectively. To perform step 4, the eyes were light-adapted three minutes at approximately 10 candle power and a photopic white 0 dB single flash ERG was performed.

**Histological Examination**

Euthanasia was performed following the final postinjection examination, 14 days after the intravitreal injection, with an intravenous dose of 100 mg/kg pentobarbital. The eyes were immediately enucleated and fixed in 2% paraformaldehyde and 3% glutaraldehyde. Following fixation for 24 hours, the eyes were hemisected and each part was dehydrated, embedded in paraffin, serial sectioned, and stained with hematoxylin-eosin for light microscopy.

**RESULTS**

**Biomicroscopy and Ophthalmoscopy**

By biomicroscopy and indirect ophthalmoscopy, no gross evidence of a toxic reaction was seen during
the 14-day period in any eye injected with 10 or 50 μg of tacrolimus. Similarly, serial exams showed normal findings in the control eyes that received intravitreal injection of balanced salt solution.

In group 3, 1 of 4 eyes that received 100 μg of the drug developed a vitreous reaction consisting of a few small white bodies moving on the vitreous. This reaction appeared within 24 hours of the injection and cleared 3 days later. Both eyes that received 250 μg developed the same vitreous reaction in the same time frame, but the small vitreous bodies persisted for 7 days after the intravitreal injection.

In group 5, all 3 eyes that received a dose of 500 μg of tacrolimus showed a lot of white vitreous opacities (Figure 1) that appeared within 24 hours and cleared 7 days later. These vitreous bodies were larger than the ones observed in the eyes that received 100 or 250 μg. Despite the presence of vitreous opacities, the vitreous body remained sufficiently clear to permit examination of the retina in all eyes. Central opacity of the posterior capsule of the lens was observed in 1 eye of this group.

All 3 eyes that received 1000 μg developed the same type of vitreous opacities observed in group 5. These vitreous opacities were still present 10 days after the intravitreal injection. Within 3 days after the injection, we observed occlusion of the temporal retinal vessels in 1 eye that progressed to complete whitening and atrophy of these vessels (Figure 2).

Electroretinography
Examination of the electroretinogram taken before intravitreal injection of the drug showed no abnormalities. By 7 days postinjection, a marked reduction in mean B-wave amplitude was noted in the 500 and 1000 μg groups in both scotopic and photopic conditions. No ERG evidence of a toxic reaction was apparent in the eyes that received 250 μg or less of tacrolimus (Figure 3).

HISTOLOGICAL FINDINGS
Histological examination by light microscopy of the control eyes and all eyes that received 10, 50, 100

Figure 3A. Electroretinograph (scotopic white 0 dB) of rabbit eyes before injection shows b-wave amplitude 302.67 μV in the right eye and 333.79 μV in the left eye.

Figure 3B. Electroretinograph (scotopic white 0 dB) of a rabbit 7 days after intravitreal injection of 250 μg/0.1 mL tacrolimus shows no effect on the b-wave amplitude (right eye, 410.45 μV; left eye, 414.34 μV).
or 250 µg of tacrolimus exhibited normal retinal structures with no evidence of a toxic reaction (Figure 4). Fibrin was present in the vitreous in all eyes receiving 500 or 1000 µg. These eyes showed mild disorganization of the retina with loss of photoreceptor cells and vacuolation of the inner nuclear layer (Figure 5).

DISCUSSION

Tacrolimus is a potent immunosuppressive agent that has a pharmacophysiologic action similar to that of cyclosporine A, suppressing lymphocytes reactions, the production of T-cell-mediated soluble factors, and the expression of interleukin-2 receptor.1 The immunosuppressive properties of tacrolimus have been confirmed in patients undergoing liver, kidney, and heart transplantations.8-10

A previous study has shown that systemic tacrolimus was effective in treating refractory uveitis, including Behcet’s disease, but it also caused a variety of adverse side effects.13 In some patients, side effects such as renal impairment, hyperglycemia, and meningitis-like symptoms required discontinuation of the therapy.11

This study is the first published work evaluating the possible ocular toxicity of intravitreal tacrolimus. Doses of 10 to 250 µg were injected into the vitreous cavity of normal rabbit eyes without causing any changes that could be detected microscopically or by
ERG. Although vitreous opacities were observed in 1 of 4 of the eyes that received 100 μg and both eyes that received 250 μg, these transient opacities that probably represent drug precipitates were reabsorbed quickly. Intravitreal doses of 500 and 1000 μg proved to be toxic to the retina, causing marked decrease in mean B-wave amplitude in the ERG. All eyes receiving these doses showed histologic toxic reactions characterized by disorganization of the retina with loss of photoreceptor cells. One eye injected with 1000 μg developed vascular occlusion. The encouraging results of this toxicity study allow further experimentation with tacrolimus in determining its effectiveness against experimentally induced uveitis.

REFERENCES


