

Long-term Retinal Toxicity of Intravitreal Commercially Available Preserved Triamcinolone Acetonide (Kenalog) in Rabbit Eyes

Thomas A. Albin, Mubammad M. Abd-El-Barr, Petros E. Carvounis, Mohan N. Iyer, Robit R. Lakhanpal, Mark E. Pennesi, Patricia Chevez-Barrios, Samuel M. Wu, and Eric R. Holz

PURPOSE. To investigate whether intravitreal Kenalog (IVTK; Bristol Meyers Squibb Company, Princeton, NJ) produces histologic or electroretinographic changes in the rabbit retina up to 3 months after injection.

METHODS. Ten Dutch-belted rabbits were injected with 4 mg/0.1 mL Kenalog in one eye and 0.1 mL physiologic salt solution (PSS) in the fellow eye. Simultaneous bilateral dark-adapted electroretinography was performed 2 weeks and 12 weeks after injection in 10 and 6 rabbits, respectively. Saturated a-wave amplitude, maximal scotopic b-wave amplitude, and individual a-wave and b-wave amplitudes of IVTK-injected and control eyes were compared at 2 and 12 weeks after injection. Light microscopy was performed on both eyes of three animals 3 months after injection. Immunohistochemistry was performed with antibodies recognizing vimentin and human alveolar macrophage (HAM)-56, markers of glial cells and macrophages, respectively.

RESULTS. No significant difference was observed in the saturated a-wave or maximal scotopic b-wave amplitudes between the PSS-injected eyes and the IVTK-injected eyes at 2 weeks ($P = 0.95$ and $P = 0.56$, respectively) and 12 weeks ($P = 0.82$ and $P = 0.17$) after injection. Light microscopy and immunohistochemistry disclosed only rare macrophages in the vitreous of IVTK-injected eyes. Retinal layers, retinal pigment epithelium, and choriocapillaris in treatment and control eyes were unremarkable.

CONCLUSIONS. No demonstrable electroretinographic or histologic changes occurred to suggest immediate or delayed widespread retinal toxicity of IVTK. (*Invest Ophthalmol Vis Sci*. 2007;48:390-395) DOI:10.1167/iovs.06-0145

Several small retrospective case series have suggested that intravitreal injection of triamcinolone acetonide (TA) may be effective in conditions associated with retinal edema or subretinal fluid, including exudative age-related macular degen-

eration,¹⁻³ diabetic macular edema,^{4,5} central retinal vein occlusion,⁶ and branch retinal vein occlusion.^{7,8} Intravitreal nonpreserved TA seems to be safe; no long-term retinal histologic or electroretinographic toxicity has been demonstrated after intravitreal injection of nonpreserved TA in rabbit eyes for up to 3 months.^{9,10}

Although nonpreserved TA may be compounded on special order, 4 mg (0.1 mL) of the commercially available preparation of preserved TA, Kenalog-40 (Bristol Meyers Squibb Co., Princeton, NJ), is commonly used. It includes the preservative benzyl alcohol and the suspending agents sodium carboxymethylcellulose and polysorbate 80. Despite widespread use in clinical practice, studies on the safety of intravitreal Kenalog (IVTK) injection have been limited and have shown contradictory results. Some studies have suggested that IVTK is safe. Two weeks after injection of vehicle alone in rabbit eyes, no histologic changes were demonstrated in one study.¹¹ In another study, not only did IVTK in rabbit eyes fail to demonstrate clear histologic or electroretinographic toxicity for the first week after injection, enhanced retinal function after injection was suggested.¹² In contradistinction, benzyl alcohol concentrations moderately higher than those used in clinical practice resulted in loss of photoreceptor outer segments, retinal pigment epithelial (RPE) proliferation, and localized vitritis limited to the inferior rabbit retina.¹³ Additionally, unequivocal reduction of electroretinographic responses with widespread histopathologic and electron micrographic changes were demonstrated 2 months after injection of triamcinolone with benzyl alcohol at an intravitreal concentration five times that obtained clinically.¹⁴

The present study was undertaken to investigate whether IVTK at an intravitreal concentration 2.6 times that of routine clinical IVTK causes functional (assessed by electroretinography) or structural (assessed by macroscopy, histopathology, and immunohistochemistry) deleterious effects over a 12-week period (five half-lives of TA) in the rabbit eye.

MATERIALS AND METHODS

Animals, Anesthesia, Injections, and Funduscopy

The experiment was carried out in accordance with ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and was approved by the institutional review board at the Baylor College of Medicine. Fifteen Dutch-belted rabbits (each weighing 2–2.5 kg) were used. Rabbits were anesthetized with intramuscular injections of 0.2 mL/100 g body weight ketamine (95 mg/mL) and xylazine (5 mg/mL). Proparacaine hydrochloride 0.5% was used for additional topical anesthesia. Each pupil was then dilated with 10% phenylephrine. All eyes underwent baseline dilated funduscopic examination. The conjunctiva was irrigated with 1 mL of 5% povidone iodine before injection. One eye of each rabbit received an intravitreal injection of 0.1 mL Kenalog-40 through a 27-gauge needle inserted superiorly 2 mm posterior to the limbus (IVTK eyes). The contralateral eye received an intravitreal

From the Cullen Eye Institute, Baylor College of Medicine, Houston, Texas.

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Corresponding author: Eric R. Holz, Cullen Eye Institute, Baylor College of Medicine, 6565 Fannin, NC-205, Houston, TX 77030; eholz@bcm.tmc.edu.

TABLE 1. Results of b-Wave and a-Wave Analysis in Intravitreal Physiologic Salt Solution (PSS)-Injected and Intravitreal Kenalog (IVTK)-Injected Eyes Two and Twelve Weeks Postinjection

Group	n	Time (wk)	$b_{\text{scot,max}} (\mu\text{V}) \pm 1 \text{ SEM}$	$a_{\text{sat}} (\mu\text{V}) \pm 1 \text{ SEM}$
Control	10	2	252.9 \pm 22	113.0 \pm 9
	6	12	180.0 \pm 18	78.0 \pm 9
IVTK	10	2	274.4 \pm 29	114.7 \pm 13
	6	12	228.0 \pm 34	81.0 \pm 8

There is no significant difference between control and IVTK eyes in saturated a-wave (a_{sat}) or maximum scotopic b-wave ($b_{\text{scot,max}}$). Results display \pm standard error of the mean (SEM).

injection of 0.1 mL physiologic salt solution (PSS) through a 27-gauge needle in the same fashion (control eyes). The first 10 animals received IVTK in the right eye; the remaining 5 animals received IVTK in the left eye. Gatifloxacin ophthalmic solution 0.3% (Allergan, Irvine, CA) was applied to the ocular surface after injection. Dilated funduscopy with a 28-D condensing lens and an indirect ophthalmoscope was performed on 10 animals at 2 and 4 weeks after injection, 8 animals at 8 weeks, and 6 animals at 12 weeks after injection.

Electroretinography

Simultaneous bilateral electroretinography was performed 2 weeks and 12 weeks after injection in 10 and 6 rabbits, respectively, as previously described.¹⁵ At both time points, half the animals tested had IVTK in the right eye and half had IVTK in the left eye. Before testing, rabbits were allowed to dark adapt for at least 1 hour. Under dim red light, rabbits were anesthetized with intramuscular injection of 0.2 mL/100 g body weight ketamine (95 mg/mL) and xylazine (5 mg/mL). Pupils were dilated with a single drop of 2.5% phenylephrine and 1% tropicamide. One drop of 0.5% proparacaine hydrochloride was applied for corneal anesthesia. Rabbits were placed inside a Ganzfeld dome coated with highly reflective white paint. A small amount of 2.5% methylcellulose gel was applied to the eye, and a contact lens electrode (JET; LKC Technology, Gaithersburg, MD) was placed in contact with the central corneal area. Platinum reference and ground electrodes (Telefactor; Grass, West Warwick, RI) were placed through the eyelid and ear, respectively. After placement in the dome, rabbits were allowed to dark adapt in complete darkness for several minutes. Signals were amplified with an amplifier (P122; Grass; bandpass, 0.1–300 Hz). Data were acquired using the National Instruments Laboratory-PC DAQ board (sampling rate, 10,000 Hz), and between 3 and 10 traces (depending on the signal-noise ratio at various light intensities) were averaged and analyzed with custom software (Matlab; Mathworks, Natick, MA). Flashes were calibrated in a manner similar to that detailed elsewhere.¹⁵ Flashes for scotopic b-wave measurements were generated by a photostimulator (PS33+; Grass). Light was spectrally filtered with a 500-nm interference filter. Flashes varied in intensity from -3.80 to $-0.76 \log \text{scot cd} \cdot \text{s/m}^2$. For analysis of the a-wave, we used a 1500-W xenon flash lamp (Novatron, San Diego, CA), which allowed the light stimuli to vary from 0.33 to $2.97 \log \text{scot cd} \cdot \text{s/m}^2$.

Euthanatization and Histology

Twelve weeks after injection, three animals were humanely killed with overdoses of intracardiac ketamine and xylazine. The eyes were enucleated, the corneas were incised, and the eyes were fixed immediately in 10% buffered formalin. Eyes were sectioned horizontally to obtain a pupil-optic nerve section and were examined macroscopically. Tissues were then processed and embedded in paraffin, sectioned at a thickness of $5 \mu\text{m}$, and stained with hematoxylin-eosin. Light microscopy was used for histologic examination. The remaining animals were humanely killed at 2, 4, 8, and 12 weeks for pharmacodynamic studies; these findings will be reported separately.

Immunohistochemistry

Paraffin sections of the rabbit eye were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol. All sections underwent antigen retrieval for 25 minutes (Citrate Target Retrieval; DakoCytomation, Carpinteria, CA), and adequate positive control was used. Primary antibody (HAM-56 diluted 1:40 and vimentin diluted 1:500; DakoCytomation) was used for 25 minutes. A detection system (LSAB+System-AP; DakoCytomation) was used with diaminobenzidine (DAB; DakoCytomation) as chromogen. Slides were counterstained with hematoxylin.

Data and Statistical Analyses

Scotopic b-wave analysis was performed by fitting light stimulus and response to a saturating hyperbolic function (Naka-Rushton) of the form:

$$b = \frac{b_{\text{max,scot}} I}{I_{0.5} + I}$$

where b is the maximum filtered b-wave, $b_{\text{max,scot}}$ is the saturating dark adapted b-wave amplitude, I is the intensity of stimulus, and $I_{0.5}$ is the half-saturating stimulus.

From such an analysis, $b_{\text{max,scot}}$ and $I_{0.5}$ could be extracted, as could the associated errors in the fit. For a-wave analysis, the maximum a-wave amplitude was recorded at each light stimulus. The a-wave amplitude recorded from a saturating light stimulus ($2.97 \log \text{scot cd} \cdot \text{s/m}^2$) was denoted as a_{sat} .

We used the paired Student t test to test our primary hypothesis that there would be no statistically significant difference between the a_{sat} and $b_{\text{max,scot}}$ of IVTK eyes and control eyes at 2 and 12 weeks. Additionally, we used paired Student t tests to compare a- and b-wave amplitudes at each intensity of stimulating light. Statistical significance was defined as $\alpha = 0.05$. Finally, we qualitatively compared funduscopy and histopathologic findings between IVTK eyes and control eyes.

RESULTS

Funduscopy

Ten animals underwent bilateral dilated fundus examination 2 and 4 weeks after injection, eight underwent it 8 weeks after injection, and six underwent it 12 weeks after injection. All animals had evidence of IVTK depot in the inferior vitreous of the IVTK eye at all time points. The size of the depot decreased with time. In all six IVTK eyes, small remnants of depot could be observed 12 weeks after injection. No keratic precipitates,

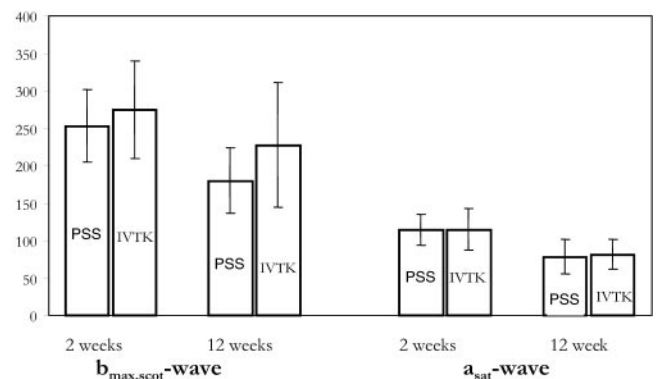


FIGURE 1. Mean or maximum scotopic b-wave ($b_{\text{max,scot}}$) and saturated a-wave (a_{sat}) amplitudes (with bars indicating $1.05 \times \text{SE}$) at 2 and 12 weeks for IVTK- and intravitreal PSS-injected eyes. No significant difference was observed between the two groups at either time point.

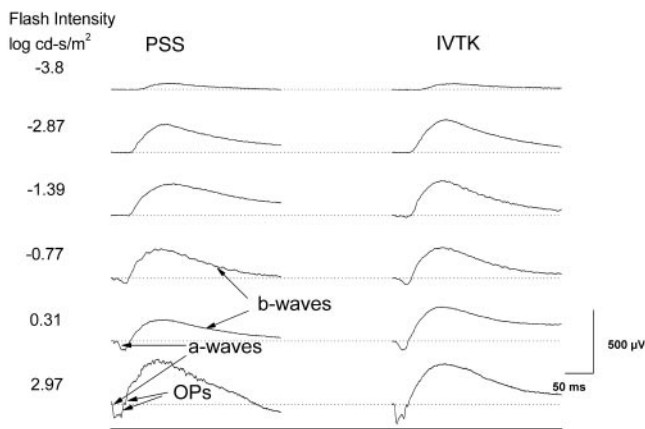


FIGURE 2. Representative scotopic electroretinogram recordings from an animal 2 weeks after IVTK injection in one eye and PSS injection in the contralateral eye at different flash intensities. Arrows indicate a-waves, b-waves, and oscillatory potentials (OPs).

hypopyon, hyphema, cataract, vitritis, or vitreous hemorrhage was observed at any time point, and no evidence of retinal or retinal pigment epithelial change was noted.

Electroretinography

No significant difference was observed in the a_{sat} or $b_{\text{max,scot}}$ between the PSS-injected eyes and the IVTK-injected eyes at 2 weeks ($P = 0.95$ and $P = 0.56$, respectively) and 12 weeks ($P = 0.82$ and $P = 0.17$) after injection (Table 1, Figs. 1 and 2). A statistically significant ($P = 0.035$) decline was observed in $b_{\text{max,scot}}$ between 2 and 12 weeks in control eyes but not in IVTK eyes ($P = 0.31$). Similarly, the decline in a_{sat} was significant in control ($P = 0.02$) but not in IVTK-injected eyes ($P = 0.07$).

We found no statistically significant difference in the a-wave or b-wave amplitudes between IVTK eyes and control eyes at 2 weeks (10 pairs of eyes tested at 16 stimulus intensities; Figs. 3A, 3C). At 12 weeks (six pairs of eyes tested at 13 stimulus intensities), no statistically significant difference in b-wave amplitudes was observed between IVTK eyes and control eyes (Fig. 3D). At a stimulating intensity of -1.77 log scot cd \cdot s/m², the a-wave amplitude was larger in IVTK eyes than in control eyes ($P = 0.02$), but no significant difference was observed in a-wave amplitudes in each of the other 12 stimulating intensities tested (Fig. 3C).

Histology

Hematoxylin-eosin-stained sections through the globes at 12 weeks disclosed normal neurosensory retina, retinal pigment

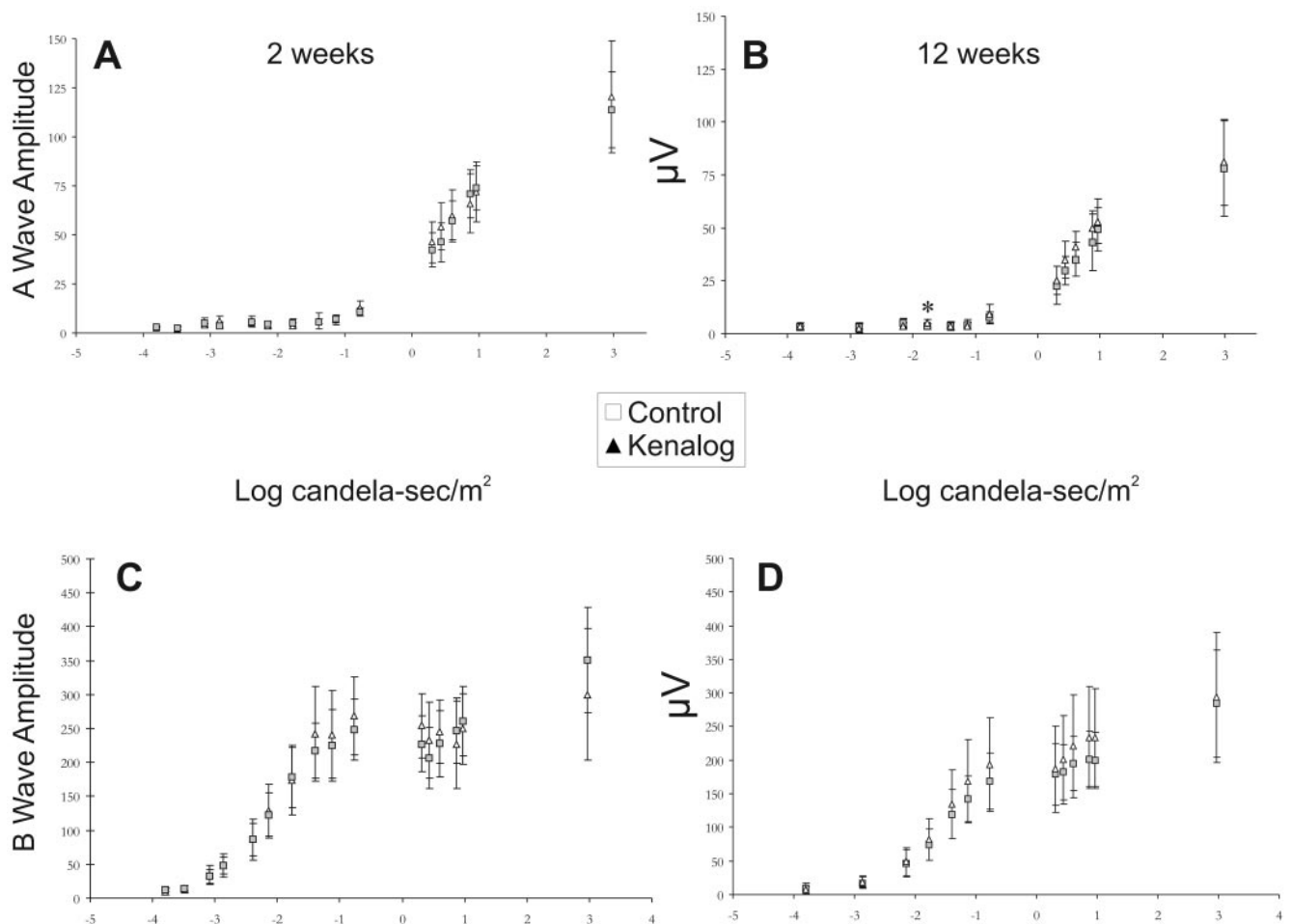


FIGURE 3. Comparisons of a- and b-wave amplitudes at 2 and 12 weeks. Mean a-wave (A, B) and b-wave (C, D) amplitudes (with bars indicating $t_{0.05} \times \text{SE}$) for IVTK eyes and control eyes 2 weeks (A, C) and 12 weeks (B, D) after injection, for 16 and 13 different stimulating intensities, respectively. None of the differences are statistically significant, except for a single stimulating intensity of -1.77 log scot cd \cdot s/m² ($P = 0.02$) at 12 weeks (B, asterisk).

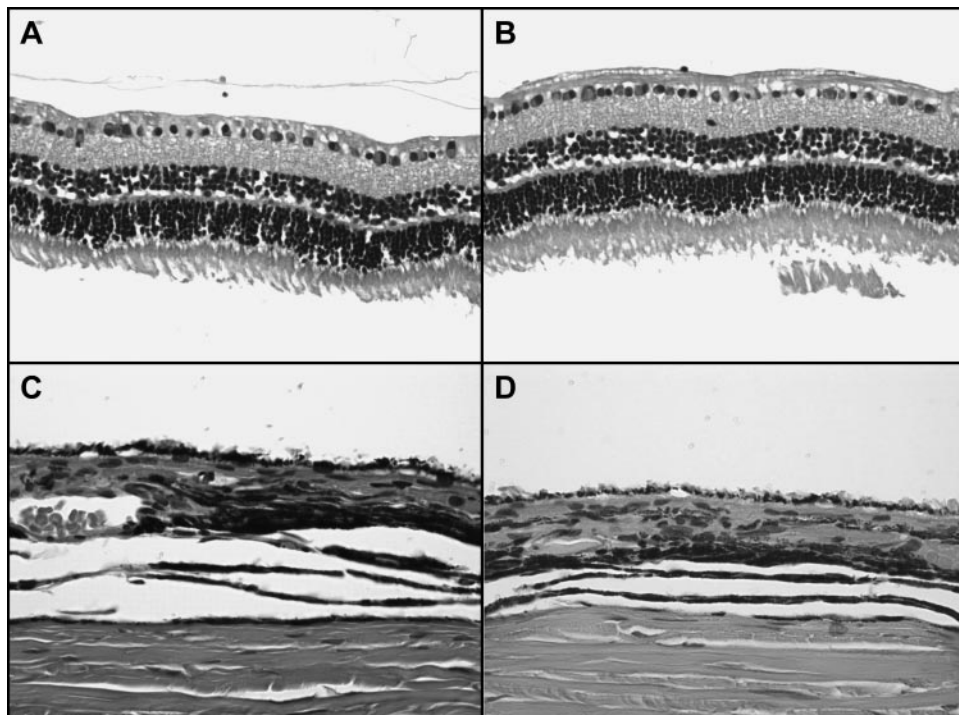


FIGURE 4. Hematoxylin-eosin-stained micrograph of retina (A, B; original magnification, $\times 20$) and retinal pigment epithelium/choriocapillaris complex (C, D; original magnification, $\times 40$) of the PSS-injected eye (A, C) and the IVTK-injected eye (B, D) in the same animal. No significant differences were noted on histologic examination.

epithelium, and choriocapillaris in both eyes of three animals (Fig. 4). In 1 of 3 IVTK and in 1 of 3 control eyes, degenerated erythrocytes were observed near the vitreous base. A few mononuclear cells were seen in IVTK eyes but not in control eyes in the vitreous. Immunohistochemistry with primary antibody (HAM-56; DakoCytomation) disclosed these cells to be macrophages. A few cells were also observed on the internal limiting membrane in a single control eye, and staining with antivimentin antibody disclosed these cells to be stain positive, consistent with astrocytes. Vimentin staining also showed diffuse expression from the internal limiting membrane to the outer nuclear layer, representing the extent of Müller cells (Fig. 5). Control and IVTK eyes displayed diffuse glial staining, suggesting normal length of Müller cells with normal foot processes.

DISCUSSION

Although a previous study found no toxicity 1 week after IVTK when used at an intravitreal concentration only 2.6 times that used in the clinical setting¹² and another study found long-term toxicity at intravitreal concentrations greater than 5 times that used in clinical practice,¹⁴ the present study sought to examine long-term toxicity at the lower intravitreal concentration of 2.6 times used in the clinical setting.

Electroretinography showed no statistically significant difference between IVTK-injected and control eyes in a_{sat} or $b_{max,scot}$ at 2 or 12 weeks after injection. Similarly, no statistically significant difference was observed in a-wave or b-wave amplitudes at a broad range of light intensities between IVTK eyes and control eyes except for an increase in IVTK eyes in a-wave amplitude for a stimulating intensity of $-1.77 \log \text{scot cd} \cdot \text{s/m}^2$ ($P = 0.02$) at 12 weeks. This difference is not statistically significant if Bonferroni correction for multiple comparisons is applied (though the validity of applying such a correction is controversial).¹⁶ We doubt this is a true effect. Rather, it most likely occurred as a consequence of performing 58 comparisons; by chance alone, 1 in 20 comparisons was found to be significant ($P = 0.05$).

Histology and immunohistochemistry examination results of pupil-optic nerve sections further confirmed a lack of photoreceptor, glial, RPE, or choriocapillaris toxicity. Specifically, no necrosis, inflammatory response, gliosis, or atrophic changes in the retinal layers were seen when comparing IVTK eyes with controls. The only other difference noted between IVTK eyes and control eyes was the presence of scattered vitreous macrophages in IVTK eyes. It is probable that these macrophages represented a phagocytic reaction involved in clearing the IVTK depot and not clinical vitreitis. The few astrocytes seen on the internal limiting membrane of a single control eye might have represented a focal response to micro-trauma of the injection.

Previous studies have reported contradictory results: though some authors claim IVTK enhances retinal function, as demonstrated by electroretinography,¹² others have found no change, and still others have found a profound decline. Dierks et al.¹² investigated the electroretinographic and histologic effects of IVTK up to 7 days after injection. At 1 of 7 stimulating intensities (i.e., at $-0.83 \log \text{scot cd} \cdot \text{s/m}^2$), they observed a statistically significant ($P = 0.02$) increase in b-wave ratio between IVTK eyes and uninjected eyes and between supernatant-injected eyes and uninjected eyes but not between any of the other groups, and they concluded that IVTK may enhance retinal function.¹² We think this enhancement likely represented a chance finding resulting from multiple comparisons, as also occurred during statistical analysis in our study.

The preponderance of data in the study by Dierks et al.¹² and in our study seem to support the conclusion that IVTK injection achieving an intravitreal benzyl alcohol concentration of 0.071% (0.1 mL of 0.99 mg/mL benzyl alcohol in 1.4 mL rabbit vitreous) does not cause diffuse retinal toxicity. Morrison et al.¹⁵ recently reported that intravitreal 0.073% benzyl alcohol results in localized areas of photoreceptor loss inferiorly at 2 weeks in the rabbit. Notably, however, accompanying electroretinography was normal at this and at a fivefold higher intravitreal concentration, suggesting either that the toxicity was significantly limited in extent or that electrophysiologic

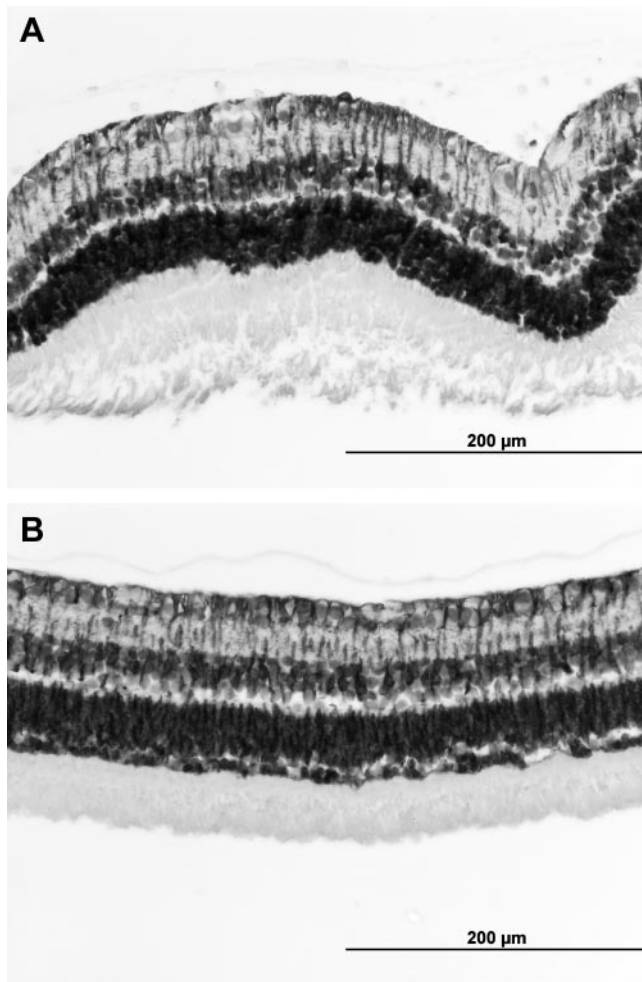


FIGURE 5. Immunohistochemistry with antivimentin antibody in the PSS-injected eye (**A**) and the IVTK-injected eye (**B**) in the same animal demonstrated diffuse vimentin expression from the internal limiting membrane to the outer nuclear layer, representing the full extent of Müller cells. Control and IVTK eyes displayed diffuse staining, suggesting Müller cells of normal length with normal foot processes.

examination in this study lacked sufficient power because of the small number of animals and the lack of controls. Given that we concentrated on pupil-optic nerve sections, we might have missed the changes described in the inferior peripheral retina by Morrison et al.¹³ Arguably, however, such changes might have resulted from higher local concentrations of benzyl alcohol and, if restricted to peripheral areas of inferior retina, may not be clinically relevant.

Kai et al.¹⁴ noted a profound decline in electroretinographic responses and associated photoreceptor outer segment loss observed on histologic examination 8 weeks after intravitreal injection of a different commercially available preserved triamcinolone product (JIDA Company, Kunming, China) with a composition similar to that of Kenalog. In that study, however, the benzyl alcohol vitreous concentration was 0.143% (more than five times the intravitreal concentration resulting from 0.1 mL IVTK injection in clinical practice). Interestingly, Morrison et al.¹³ did not observe electroretinographic changes at vitreous concentrations exceeding 0.143% benzyl alcohol 2 weeks after injection; it is therefore possible that some of the electroretinographic findings noted by Kai et al.¹⁴ represented delayed toxicity. In our study, such delayed toxicity (more than five half-lives of TA in rabbit eyes [<3

days]^{17,18} and roughly equivalent to five reported half-lives of IVTK in humans [18.6 days])¹⁹ did not occur at the benzyl alcohol vitreous concentration of 0.071% (2.6 times that in human vitreous after 0.1 mL IVTK injection). This is an important observation because Kai et al.¹⁴ conclude that the preservative in their drug causes widespread retinal toxicity; we found no evidence of widespread toxicity at a more clinically relevant concentration.

Numerous limitations of our study must be considered. The small number of animals used (10 for week 2 electroretinography, 6 for week 12 electroretinography, 3 for histology) makes it possible that a real difference was not detected (type 2 statistical error). Because no statistically significant difference in electroretinography between IVTK and control eyes was found in this study, careful attention to the power of the study is appropriate. Assuming that a clinically insignificant change in electroretinography would be less than a 20% difference, we would have needed at least 34 animals given the observed measurement variance to achieve 0.9 power of detecting a difference at the $\alpha = 0.05$ level. Surprisingly, some vitreous opacity on funduscopy interpreted as depot and intravitreal macrophages were present in all IVTK-injected eyes 3 months after injection, though the published half-life of TA after intravitreal injection is less than 3 days in the rabbit.^{17,18} It is unclear whether the observed depot represents persistent drug or localized vitreous opacification caused by phagocytic response. A separate study to determine vitreous concentrations of IVTK over 3 months in the same animals is in progress. Some IVTK toxicity studies have used pigmented rabbits,^{9,11} and others have used New Zealand White rabbits.^{10,12-14} The effects of ocular pigmentation were not specifically addressed by our study. In contrast to the clinical situation, the retinas in these animals were unaffected by disease (it may be that diseased retina and retinal pigment epithelium are more susceptible to cytotoxicity). Dark-adapted electroretinography does not assess cone function, and the rabbit fovea is not identical to the human fovea. This study does not address concerns about sterile inflammation after IVTK, termed pseudo-endophthalmitis, thought to be an immune response to the vehicle; large, clinical comparative studies of Kenalog and preservative-free TA are required to adequately address this concern. As mentioned previously, results of gross examination of the retina were normal; therefore, histologic examination was confined to pupil-optic nerve sections. Consequently, we cannot comment on the presence or absence of localized photoreceptor damage outside these sections.

In conclusion, IVTK resulting in a final benzyl alcohol concentration of 0.071% (more than twice that achieved in clinical practice after 0.1 mL IVTK injection) produced no demonstrable deleterious electroretinographic effects or any histopathologic or immunohistochemical changes on pupil-optic nerve sections even 3 months after IVTK injection. The finding of localized vitreous opacity inferiorly and of intravitreal macrophages in all IVTK-injected eyes 3 months after injection may indicate persistent drug or localized vitreous opacification because of the phagocytic response, and it underscores the importance of long follow-up to ensure the absence of delayed toxicity. This study does not support or contradict the finding of peripheral focal photoreceptor loss from an identical concentration of benzyl alcohol, as described by Morrison et al.¹³; however, normal histologic examination findings on the pupil-optic nerve sections (which in clinical practice would correspond to the macula) suggest these findings may not be clinically relevant. Toxicity studies, performed in higher animals, of benzyl alcohol administration with or without triamcinolone and with particular attention to macular and peripheral retinal changes may be more reliably extrapolated to hu-

mans and may further establish the safety and potential risks of IVTK.

References

1. Challa JK, Gillies MC, Penfold PL, et al. Exudative macular degeneration and intravitreal triamcinolone: 18 month follow up. *Aust N Z J Ophthalmol*. 1998;26:277-281.
2. Spaide RF, Sorenson J, Maranan L. Photodynamic therapy with verteporfin combined with intravitreal injection of triamcinolone acetonide for choroidal neovascularization. *Ophthalmology*. 2005;112:301-304.
3. Danis RP, Ciulla TA, Pratt LM, Anliker W. Intravitreal triamcinolone acetonide in exudative age-related macular degeneration. *Retina*. 2000;20:244-250.
4. Martidis A, Duker JS, Greenberg PB, et al. Intravitreal triamcinolone for refractory diabetic macular edema. *Ophthalmology*. 2002;109:920-927.
5. Jonas JB, Akkoyun I, Kreissig I, Degenring RF. Diffuse diabetic macular oedema treated by intravitreal triamcinolone acetonide: a comparative, non-randomised study. *Br J Ophthalmol*. 2005;89:321-326.
6. Ip MS, Gottlieb JL, Kahana A, et al. Intravitreal triamcinolone for the treatment of macular edema associated with central retinal vein occlusion. *Arch Ophthalmol*. 2004;122:1131-1136.
7. Jonas JB, Akkoyun I, Kampeter B, Kreissig I, Degenring RF. Branch retinal vein occlusion treated by intravitreal triamcinolone acetonide. *Eye*. 2005;19:65-71.
8. Flynn HW Jr, Scott IU. Intravitreal triamcinolone acetonide for macular edema associated with diabetic retinopathy and venous occlusive disease: it's time for clinical trials. *Arch Ophthalmol*. 2005;123:258-259.
9. McCuen BW 2nd, Bessler M, Tano Y, Chandler D, Machermer R. The lack of toxicity of intravitreally administered triamcinolone acetonide. *Am J Ophthalmol*. 1981;91:785-788.
10. Kivilcim M, Peyman GA, El-Dessouky ES, Kazi AA, Cheema R, Hegazy H. Retinal toxicity of triamcinolone acetonide in silicone-filled eyes. *Ophthalmic Surg Lasers*. 2000;31:474-478.
11. Hida T, Chandler D, Arena JE, Machermer R. Experimental and clinical observations of the intraocular toxicity of commercial corticosteroid preparations. *Am J Ophthalmol*. 1986;101:190-195.
12. Dierks D, Lei B, Zhang K, Hainsworth DP. Electroretinographic effects of an intravitreal injection of triamcinolone in rabbit retina. *Arch Ophthalmol*. 2005;123:1563-1569.
13. Morrison VL, Koh HJ, Cheng L, Bessho K, Davidson MC, Freeman WR. Intravitreal toxicity of the kenalog vehicle (benzyl alcohol) in rabbits. *Retina*. 2006;26:339-344.
14. Kai W, Yanrong J, Xiaoxin L. Vehicle of triamcinolone acetonide is associated with retinal toxicity and transient increase of lens density. *Graefes Arch Clin Exp Ophthalmol*. 2006;244:1152-1159.
15. Hua G, Pennesi M, Shah K, et al. Safety of intravitreal voriconazole: electroretinographic and histopathologic studies. *Trans Am Ophthalmol Soc*. 2003;101:183-189.
16. Perneger T. What's wrong with Bonferroni adjustments? *BMJ*. 1998;316:1236-1238.
17. Chin HS, Park TS, Moon YS, Oh JH. Difference in clearance of intravitreal triamcinolone acetonide between vitrectomized and nonvitrectomized eyes. *Retina*. 2005;25:556-560.
18. Scholes GN, O'Brien WJ, Abrams GW, Kubicek MF. Clearance of triamcinolone from vitreous. *Arch Ophthalmol*. 1985;103:1567-1569.
19. Beer PM, Bakri SJ, Singh RJ, Liu W, Peters GB 3rd, Miller M. Intraocular concentration and pharmacokinetics of triamcinolone acetonide after a single intravitreal injection. *Ophthalmology*. 2003;110:681-686.