Retinal Toxicity of Indocyanine Green in Albino Rabbits

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PURPOSE. Intravitreal indocyanine green (ICG) is commonly used in vitreoretinal surgery. The purpose of this study was to evaluate possible toxicity of ICG in the retina of albino rabbits.

METHODS. Twenty-two albino rabbits were injected intravitreally with 0.1 mL ICG solution in one eye, and three rabbits were studied for the effects of 0.1 mL distilled water. All rabbits were injected intravitreally with 0.1 mL saline into the fellow eye, which served as the control. The electroretinogram (ERG) and visual evoked potential (VEP) were recorded from each rabbit at different time intervals after injection. The rabbits were killed at the termination of the follow-up periods and their retinas prepared for histologic examination at the light microscopic level.

RESULTS. Three hours after injection, the ERG responses were reduced in amplitude in all ICG-injected eyes, and the VEPs were of abnormal pattern (reduced amplitude and delayed). Partial dose-dependent recovery was observed during 4 weeks of follow-up. Light microscopy of the retinas of the experimental eyes exhibited considerable damage to all retinal layers in all eyes studied that received the highest ICG dose.

CONCLUSIONS. ICG is potentially toxic to all retinal layers of the albino rabbit. Although it is difficult to extrapolate these findings directly to human eyes, caution should be exercised when using ICG intravitreally. (*Invest Ophthalmol Vis Sci.* 2006;47: 2100–2107) DOI:10.1167/iovs.05-0206

Intravenous administration of indocyanine green (ICG) is commonly used in the ophthalmic clinic for detecting disease in the choroidal vasculature.[1-3] In the past few years, intravitreal administration of ICG during vitrectomy has been suggested for idiopathic macular hole or for retinal detachment secondary to myopic macular hole.[4-11] The dye selectively stains the internal limiting membrane (ILM),[5,7] improving visualization and facilitating removal of the ILM without injuring the underlying unstained retina.

Despite the surgical benefits of ICG staining and the successful anatomic results of its use, recent reports have demonstrated unsatisfactory functional outcome. Visual acuity was poorer in eyes treated with ICG compared with those of control subjects,[12-14] and visual field defects were found after surgery in eyes that had undergone ICG-assisted peeling of the ILM during vitrectomy surgery.[13-15] Twelve months of follow-up that included electroretinography (ERG), optical coherence tomography (OCT), and fluorescein angiography mea-surements of patients who underwent ILM peeling with ICG validated the presence of ICG-associated damage to ganglion cells and their axons in addition to retinal pigment epithelial (RPE) damage.[16] Funduscopic findings in eyes after ILM peeling during macular hole surgery revealed unusual RPE atrophy in areas where the cells were in direct contact with ICG.[17] These and other studies[18,19] raised the possibility that ICG is toxic to the retina, and prompted toxicity studies in human cadaveric eyes and animal models.

Histologic examination of retinas of eyes undergoing ICG-assisted removal of ILM have demonstrated cleavage planes within the innermost retinal layers.[20] Experimental macular surgery using ICG in donor eyes has shown that ICG alone causes rupture of Müller cells with detachment of the ILM. This ICG-induced damage is exacerbated if the retina is exposed to light containing wavelengths longer than 620 nm.[21] Several studies have demonstrated ICG toxicity to human RPE cells in vitro that could cause cell-cycle arrest and apoptosis in the presence of acute illumination.[22,23] Toxicity studies in animal models have indicated ICG toxicity in different retinal structures. Whereas one study in a porcine model of eyes that had been obtained 5 hours after enucleation and exposed to different concentrations of ICG showed no histologic damage,[24] another study showed ICG-induced RPE atrophy in a porcine model of retinal hole.[25] ICG-injected subretinally in rabbits resulted in severe, dose-dependent morphologic damage to the outer retina and photoreceptors.[26,27] In two studies involving electroretinogram (ERG) recording to test retinal function, ICG injected intravitreally led to permanent reduction in retinal function.[28,29] ICG has been found to induce functional damage in the rat retina without any apparent morphologic damage, even with a low dose of the dye (0.025 mg/mL).[28] Finally, ICG toxicity to rat ganglion cells has been documented in vitro and in vivo.[30] This observation is of extreme importance, since, in ophthalmic surgery, ICG is applied to the surface of the retina from the vitreal side, and the first cells to have direct contact with the drug are the retinal ganglion cells.

The purpose of the present study was to evaluate possible toxicity of different concentrations of intravitreally injected ICG in a rabbit model. To test possible ICG toxicity to the ganglion cells and to distal retinal layers, we recorded respectively the visual-evoked potentials (VEPs) and the electroretinogram (ERG).

METHODS

Animals

Twenty-five adult albino rabbits weighing 2.5 to 3.0 kg each were included in the study. The rabbits were housed in a 12-hour light–dark cycle and were allowed free access to water and food. All the experimental procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and to institutional guidelines.

Before intravitreal injection and electrophysiological recordings, the rabbits were anesthetized by an intramuscular injection (0.5 mL/kg body weight) of a mixture containing ketamine hydrochloride (10 mg/mL), acepromazine maleate solution (10%), and xylazine solution (2%) at a ratio of 1:0.2:0.3. Topical anesthesia (benoxinate HCl 0.4%) was administered to reduce the animal's discomfort. The pupils were fully dilated with cyclopentolate hydrochloride 1%.
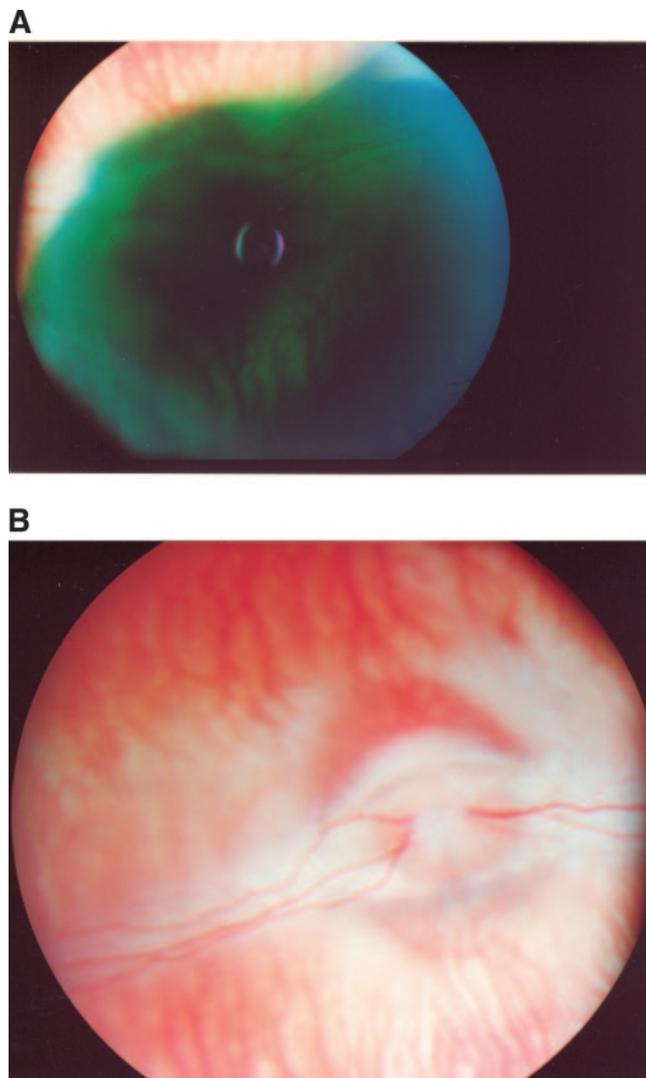
FIGURE 1. Fundus picture of the experimental (**A**) and control (**B**) eyes, 5 minutes after injecting 0.1 mL ICG solution (2.5 mg/mL) into the vitreous of the experimental eye and 0.1 mL saline into the vitreous of the control eye.

The rabbits underwent clinical inspection by indirect ophthalmoscopy and ERG and VEP recordings. The ERG and VEPs were recorded from each rabbit before intravitreal injection; 3 hours after injection, to detect immediate functional damage to the retina; and 1 week and 4 to 6 weeks after injection, to determine possible permanent damage to the retina. After the last ERG recording session, the rabbits were killed by intravenous injection of an overdose of pentobarbital sodium (80 mg/kg body weight), and their retinas were prepared for histologic examination at the light microscopic level.

Indocyanine Green

ICG was prepared according to the manufacturer's instructions (Akron, Buffalo Grove, IL) by dissolving 25 mg powder in 10 mL distilled water. The osmolarity of the solution was 7 mOsM. The rabbits were divided into four groups according to the injected solution. The first group ($n = 9$) was injected with 0.1 mL ICG solution, having a concentration of 2.5 mg/mL (original vial concentration). Rabbits in the second group ($n = 6$) were tested for the effects of 1.25 mg/mL ICG solution (0.1 mL), and those in the third group ($n = 7$) with a concentration of 0.5 mg/mL (0.1 mL). The fourth group ($n = 3$) served as a control for the effects of osmolarity on retinal function. The rabbits belonging to this group were injected with 0.1 mL distilled water in one eye. All 25 rabbits were injected intravitreally with 0.1 mL saline into the left eye, which served as control.

Intravitreal Injection

Each rabbit was injected intravitreally in both eyes as described before.[31] One eye, usually the right one, was injected with the experimental solution (ICG or distilled water) and the other with saline (control). A 25-gauge needle attached to a 1.0-mL tuberculin syringe was inserted into the vitreous approximately 1 mm posterior to the limbus. The syringe was directed under visual control with an indirect ophthalmoscope (Neitz Instruments, Tokyo, Japan) toward the center of the vitreous above the optic disc. A volume of 0.1 mL was then slowly injected.

Electroretinogram

Flash ERG responses were recorded[31] from the experimental and control eyes, by using corneal electrodes (Medical Workshop, Groningen, The Netherlands). The reference and ground electrodes were made of stainless-steel surgical needles and were inserted into the ears. The ERG signals were amplified ($\times20,000$) and filtered (0.3–300 Hz) by differential amplifiers (Grass-Telefactor, West Warwick, RI). Light stimuli were obtained from a Ganzfeld light source (LKC Technologies, Gaithersburg, MD) with a maximum intensity of 5.76 cd·s/m^2.

The ERG responses were recorded in the dark-adapted state (at least 3 hours in darkness) and then in the light-adapted state (background illumination of 1.15 cd/m^2). Six responses elicited by identical flashes applied at 10-second intervals were averaged in the dark-adapted state, and 15 responses elicited at a frequency of 1 Hz were averaged in the light-adapted state.

ERG analysis was based on measurements of the b-wave amplitude from the trough of the a-wave to the peak of the b-wave. The b-waves of the experimental and control eyes were plotted as a function of log flash intensity for each rabbit. The response-intensity curve of the ERG b-wave was fitted to a Michaelis-Menten–type hyperbolic function[32]

$$V/V_{max} = I/(I + \sigma)$$

where V is the amplitude of the ERG b-wave elicited by a stimulus of intensity I, V_{max} is the maximum response amplitude, and σ is the semisaturation constant. Functional damage in the experimental eye was assessed from the V_{max} ratio (experimental/control), and the difference in log σ (experimental − control).[31] With this approach, technical factors, such as depth of anesthesia and duration of dark adaptation did not affect the assessment of ICG-induced damage.

Visual-Evoked Potentials

The VEPs were recorded with a stainless-steel needle acting as the active electrode that was inserted under the skin above the area of the visual cortex, midway between the two ears. The reference and ground electrodes were inserted in the ears. The signal was amplified ($\times500,000$) and filtered (1–100 Hz) by a differential amplifier (Grass-Telefactor). With this electrode configuration, monocular light stimuli yielded very similar VEPs in nontreated animals. Fifty stimuli were delivered at a rate of 1.1 Hz, and the resultant signals were digitized and averaged by the computer. To minimize possible contamination of the VEPs due to light absorbed by the ICG dye, we elicited the VEPs with the brightest light stimuli 5.76 cd·s/m^2 available in our photostimulator (PS22; Grass-Telefactor).

The VEP responses were assessed quantitatively from their temporal pattern and amplitudes. The most easily identifiable waves of the flash VEP in rabbits were an initial negative wave that was followed by a prominent positive wave. The VEP amplitude was measured from the trough of the first negative wave to the peak of the following positive wave. Temporal properties of the VEP were defined by the time interval from stimulus onset to the trough of the first negative wave, termed the implicit time.
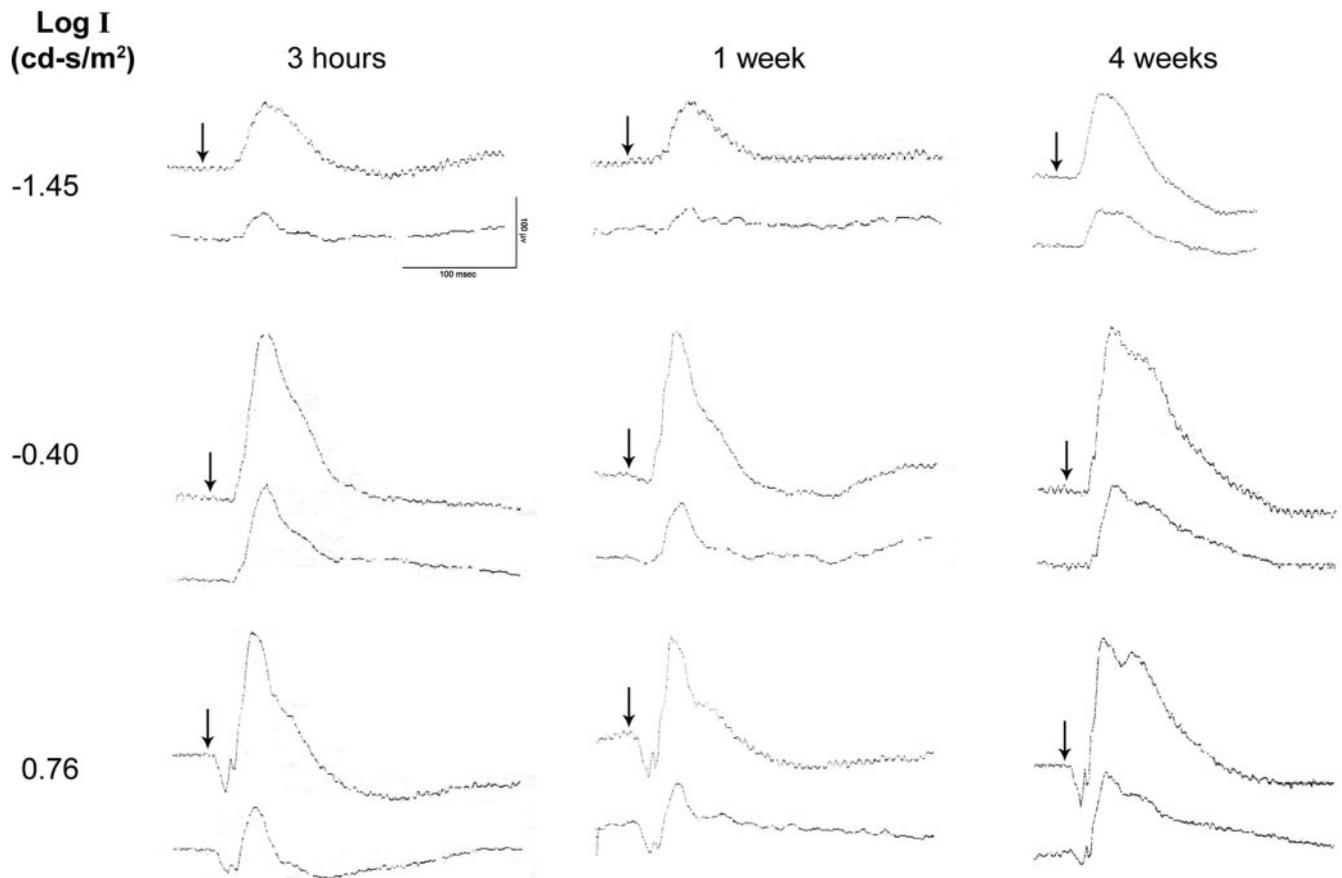
FIGURE 2. ERG follow-up of one albino rabbit that was injected with the highest ICG dose (0.25 mg). Dark-adapted ERG responses were elicited by light stimuli of different intensities as denoted in log units of cd-s/m^2 to the left of each row of responses. ERG responses were recorded 3 hours, 1 week, and 4 weeks after ICG injection. Each pair of responses compares the ERG of the experimental eye to that of the control eye (*bottom and top traces,* respectively). *Arrow:* timing of the light stimulus.

Statistical Analysis

The ERG parameters (V_{max} and σ) and the VEP parameters (amplitude and implicit time) were first tested for dependency of variance on the mean by using the Levene test for homogeneity of variance. Then, the parameters were tested for statistical significance by using the mixed model of analysis of variance with repeated measures that could be used for incomplete data points.

Histologic Examination

Histologic observations seeking abnormal retinal structures were conducted at the light microscopic level.[31] The enucleated eye was soaked for 10 minutes in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M of phosphate buffer (pH 7.4). The eyeball was opened 2 mm posterior to the limbus (pars plana) and fixed for 72 hours. After the lens and the vitreous were removed, the posterior eye cup was bi-

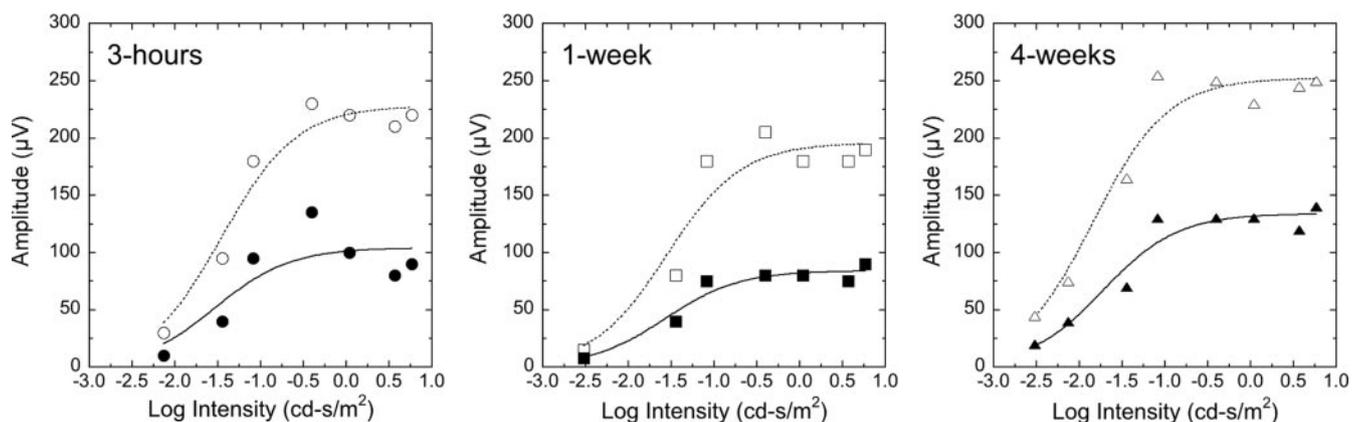

FIGURE 3. Response–intensity data for the dark-adapted ERG b-wave were measured in same rabbit as shown in Figure 2. ERG data of the experimental and control eyes are compared (*filled* and *open symbols,* respectively). The data were fitted to a Michaelis-Menten hyperbolic relationship (see the equation; *dotted curve* for the control eye and a continuous curve for the experimental eye).

TABLE 1. Maximum b-Wave Amplitude (V_{max}) and Semi-Saturation Constant (σ)

	V_{max} (exp)	V_{max} (control)	V_{max} ratio	Log σ (exp)	Log σ (control)	ΔLog σ
3 Hours	104	228	0.46	−1.53	−1.45	−0.08
1 Week	84	196	0.43	−1.58	−1.55	−0.03
4 Weeks	134	252	0.53	−1.72	−1.84	0.12

Data are from the rabbit for which the electroretinogram responses and response–intensity data are shown in Figures 2 and 3, respectively. Indocyanine green toxicity was calculated from the V_{max} ratio and the difference in log σ. V_{max} is expressed in microvolts and σ in cd-s/m^2.

sected at the level of the optic disc. One half of the eye cup was rinsed in PBS and dehydrated twice in 70% alcohol for 3 hours and twice in 96% alcohol for another 3 hours. Embedding was performed in resin (JB-4; Bio-Rad, Watford City, UK). The tissue was cut into 2-μm sections (Reichert-Jung, Nussloch, Germany), and mounted on slides. The sections were stained with Richardson's solution for examination with the light microscope.

RESULTS

Clinical Observation

After intravitreal injection, ICG appeared as a floating green mass in the vitreous (Fig. 1A), whereas the eye injected with saline (Fig. 1B) appeared clear. During the follow-up period, pigmented particles were observed floating in the vitreous and on the retinal surface of the ICG-injected eye for 4 to 6 weeks, until they completely disappeared. Fundus examination performed 4 weeks after injection, detected no apparent changes.

Electroretinogram

Figure 2 shows representative ERG responses of one rabbit that were recorded at three time intervals (3 hours, 1 week, and 4 weeks) after intravitreal injection of 0.1 mL of the original 2.5 mg/mL vial solution (making an injected ICG dose of 0.25 mg). Dark-adapted ERG responses that were elicited by flashes of different intensities (denoted in log units to the left of each row) are shown for each recording session. The ERG of the experimental eye (bottom trace) is compared to the ERG of the control eye (top trace) in each pair of responses. The ERG responses in the experimental eye were reduced by approximately 50%, compared with the control eye when measured 3 hours after injection. The ERG responses of the rabbit in Figure 2 did not change appreciably during the 4-week follow-up period.

The pattern of the ICG effect and its dependency on time after injection are better illustrated by analyzing the response–

intensity relationships. Figure 3 shows the response–intensity relationships for the dark-adapted b-waves of the experimental and control eyes of the same rabbit for which ERG responses are shown in Figure 2. The response–intensity data were fitted to the Michaelis-Menten hyperbolic function (see the equation) to derive the maximum b-wave amplitude (V_{max}) and semisaturation constant (σ), summarized in Table 1. To assess the effects of ICG on the functional integrity of the experimental eye, we calculated the V_{max} ratio (experimental eye/control eye) and the difference in the log σ. These values indicated a reduction of ~50% in V_{max} throughout the 4 weeks of follow-up and a very small change in the semisaturation constant.

ERG recordings were conducted in all 22 rabbits at each period of follow-up (3 hours, 1 week and 4 weeks after injection). We derived the response–intensity curve and calculated the V_{max} ratio and the log σ difference as discussed earlier. Figure 4 summarizes the V_{max} ratio (mean ± SD) of the three groups of rabbits at each postinjection time. Follow-up lasted for 6 weeks in some rabbits, but their data were combined with those obtained at 4 weeks, since there were no major changes in the ERG responses past 4 weeks after ICG injection.

There was a reduction of V_{max} ratio in all three groups of rabbits by 3 hours after injection. Partial recovery of the ERG responses was observed during 4 weeks of ERG follow-up. The extent of recovery depended on the injected dose. At 1-week after injection, there was no detectable change in the V_{max} ratio in the rabbits injected with a dose of 0.25 mg, but improvement was demonstrated in the other two groups (injected with 0.125 and 0.05 mg). At the end of the follow-up period (4–6 weeks), the group treated with the lowest dose (0.05 mg) of ICG exhibited close to complete ERG recovery. The other two groups also showed recovery in their ERG recordings, but did not return to control levels.

Statistical analysis showed no interaction between time and treatment, between dose and treatment and between time and dose. Therefore, we were able to apply the statistical test to each dose group of rabbits separately. There was no significant
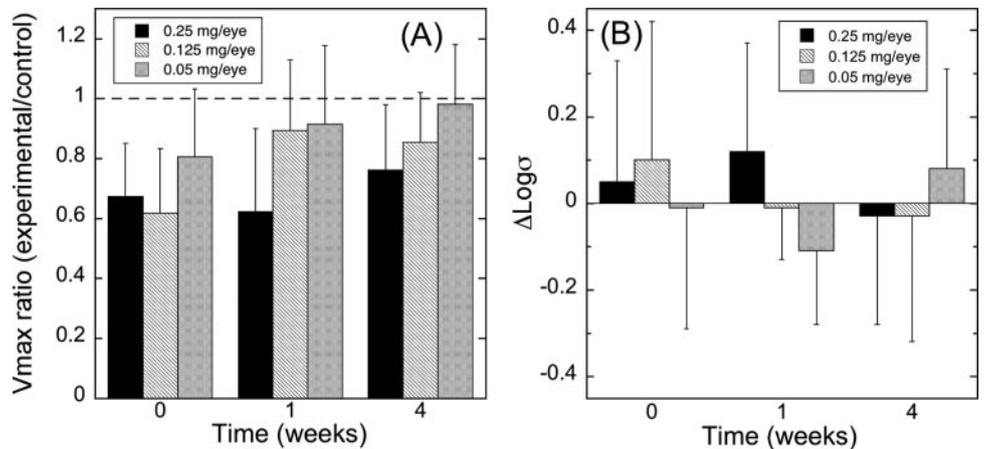

FIGURE 4. ERG follow-up of ICG-induced toxicity in three groups of albino rabbits that were injected with 0.25 ($n = 9$), 0.125 ($n = 6$), or 0.05 ($n = 7$) mg ICG. (**A**) V_{max} ratio (experimental eye/control eye) was calculated for each rabbit at each ERG recording session. The ratios in the rabbits in each group were averaged for each testing session, to assess ICG toxicity. (**B**) The effects of ICG on σ were calculated from the difference between log σ of the experimental eye and log σ of the control eye.

TABLE 2. The Effects of 0.1 mL Distilled Water on the ERG Parameters (V_{max} and Log σ) of Three Albino Rabbits

	1 Day	1 Week	4 Weeks
V_{max} ratio	1.06 ± 0.09	1.05 ± 0.07	1.06 ± 0.09
$\Delta \log \sigma$	-0.06 ± 0.13	0.10 ± 0.04	-0.06 ± 0.13

Saline (0.1 mL) was injected into the vitreous of the fellow eye, which served as the control. Mean V_{max} ratio $\pm$ SD and mean difference in log $\sigma \pm$ SD are given for 4 weeks of follow-up.

interaction between treatment and time for any of the ERG parameters. We found significant reduction in the maximum response amplitude (V_{max}) for the highest tested dose ($P = 0.0004$) and for the medium dose ($P = 0.0202$), but not for the lowest dose. The effects of ICG on the semisaturation constants of the b-wave response–intensity curves (Fig. 4B) were negligible in all three groups of rabbits, throughout the entire follow-up period.

Three rabbits that were tested for the effects of distilled water exhibited no ERG deficit throughout the entire period of follow-up (4 weeks). Mean V_{max} ratio and mean difference in log σ are listed in Table 2.

Visual-Evoked Potentials

Because ICG penetrated the retina from the vitreal side, the first cells to be exposed to it were the ganglion cells. We recorded the VEPs to test possible ICG toxicity to the ganglion cells and/or to the nerve fiber layer.

Figure 5 shows VEP responses elicited by monocular stimulation of the control and experimental eyes (top and bottom traces, respectively) of one rabbit that were recorded at 3 hours, 1 and 4 weeks after intravitreal injection of 0.25 mg ICG. The typical pattern of a negative wave appearing 40 to 60 ms after the light stimulus, followed by a positive wave, was seen in all recordings. However, the implicit time of the first negative wave is prolonged, and the amplitude of the positive wave is reduced in the experimental eye compared with the VEP of the control eye. Similar VEP responses were recorded in all the other rabbits of the three experimental groups.

We measured the implicit time of the first negative wave and the amplitude of the positive wave of the VEP responses of all the rabbits, at each period of testing, and calculated the amplitude ratio (experimental/control) and the implicit time difference (experimental − control). Figure 6 summarizes the mean ($\pm$SD) of the VEP amplitude ratio (Fig. 6A) and implicit time difference (Fig. 6B) in the three groups of rabbits. In all rabbits, regardless of ICG dose, the VEP amplitude in the experimental eye remained substantially lower than that in the control eye during the entire follow-up period, whereas the implicit time recovered toward that of the control eye after 4 weeks. Statistical analysis revealed nonsignificant interaction between treatment and time for all three dose-groups for either of the VEP parameters. The VEP amplitude was significantly reduced in the treated eye compared with the control eye for all doses throughout the follow-up period ($P = 0.0003$, 0.0202, 0.0144 for the highest, medium, and lowest doses, respectively). Significant prolongation of the implicit time for all periods of follow-up was found for the highest ($P = 0.0039$) and medium ($P = 0.0222$) doses, but not for the lowest dose.

Histologic Findings

Histologic findings of retinas from two representative rabbits that were injected with the original vial concentration of ICG, receiving the highest (0.25 mg) or the lowest (0.05 mg) doses are demonstrated in Figure 7. All four micrographs were taken close to the site of injection. The retina of the experimental eye of the rabbit treated with the highest ICG dose (bottom left) exhibited considerable damage to all retinal layers, when compared with the control retina (bottom right). The outer nuclear layer (ONL) was very thin and contained only two rows of nuclei. The inner nuclear layer (INL) was also thinner than in the control retina and only a few ganglion cells were identifiable (bottom row). The retinas from the experimental and control eyes of the rabbit injected with the lowest dose of ICG (0.05 mg), appeared similar under the light microscope, suggesting no apparent damage to retinal morphology.
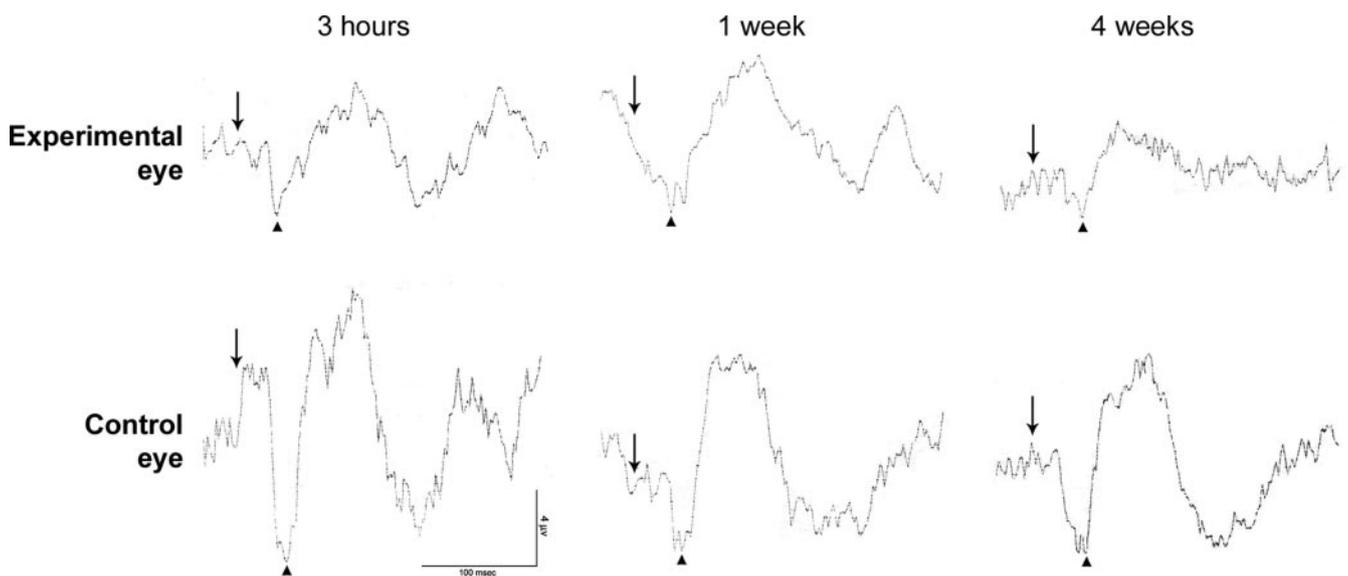

FIGURE 5. Monocular VEP responses of one albino rabbit that was injected in the experimental eye with the highest dose of ICG (0.25 mg). Flash VEP responses were elicited by bright white light stimuli that were applied to the experimental eye or to the control eye (*top* and *bottom rows*, respectively). Timing of light stimuli and of the first negative wave is denoted for each VEP response (*downward arrow* and *arrowhead*, respectively).
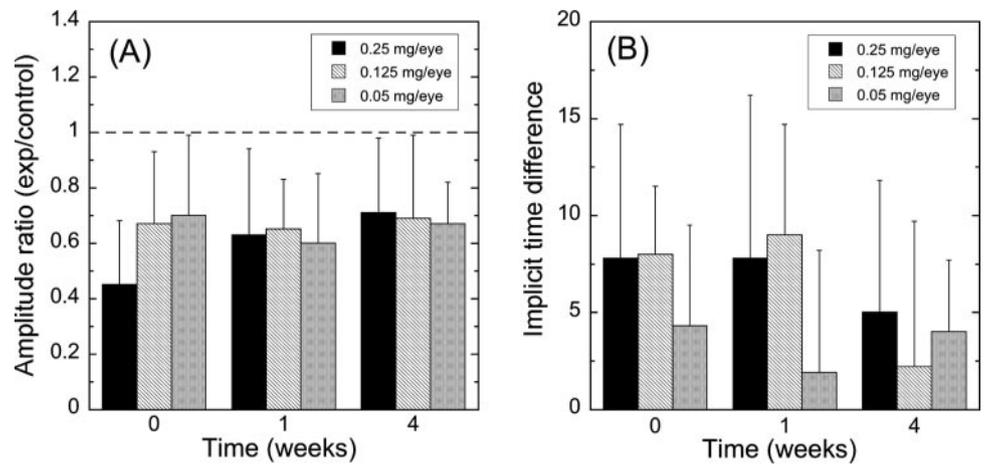
FIGURE 6. VEP follow-up of ICG-induced toxicity in three groups of albino rabbits that were injected with 0.25 ($n = 9$), 0.125 ($n = 6$), or 0.05 ($n = 7$) mg ICG. Amplitude ratio (**A**) and implicit time difference (**B**) were calculated for each rabbit in each recording session and averaged.

DISCUSSION

The electrophysiological and histologic findings in our study demonstrated dose-dependent functional and structural damage to the retina of albino rabbits by intravitreal injection of ICG. ICG exerted toxic effects on all retinal structures. The affected ERG responses indicated damage to the distal retina, and the abnormal VEPs were suggestive of damage to the proximal retina. Functional damage by ICG was evident early after injection (3 hours). The ERG responses were reduced in amplitude (Figs. 2, 3, 4), and the VEP responses were of subnormal amplitude and prolonged implicit time (Figs. 5, 6). Some of the effects of ICG were transient, with recovery being apparent within 4 weeks of follow-up, but permanent retinal damage developed with the highest two doses of ICG.

Immediately after injection, ICG appeared as a green mass floating in the vitreous (Fig. 1A). It absorbed some of the incident light, thus reducing the light intensity reaching the retina and causing the ERG responses to be reduced in amplitude. If the ICG had acted only as an optical filter, interposed in the light path, the response–intensity relationship of the ERG b-wave would be expected to be characterized by a normal maximum response (V_{max}) and an increased semisatu-

ration constant (σ). This was not the case. In every rabbit, the V_{max} of the b-wave was reduced by ICG, whereas the semi-saturation constant (σ) hardly changed (Fig. 4). Furthermore, we measured the transmittance of ICG solutions using all the concentrations that were studied. With the highest concentration (2.5 mg/mL), the transmittance between 450 to 600 nm was approximately 40% and lower at wavelengths shorter than 450 nm or longer than 600 nm, indicating only a small effect on the light impinging on the retina. This low effect is supported by the visibility of retinal blood vessels through the ICG dye in the vitreous. Thus, the early effects of ICG on the ERG responses probably reflected reduced retinal function and not optical interference. It should also be noted that a permanent ERG deficit was observed after 4-weeks of follow-up, when most of the ICG had cleared from the vitreous, supporting the notion of ICG-induced retinal damage.

The flash ERG reflects mainly light-induced activity in the distal retina, with minor contributions by light-induced activity in the proximal retina. Blocking activity in the proximal retina by specific antagonists leads to augmentation of the ERG.[33] Therefore, damage to the proximal retina is expected to cause the ERG responses to increase in amplitude or at least not to
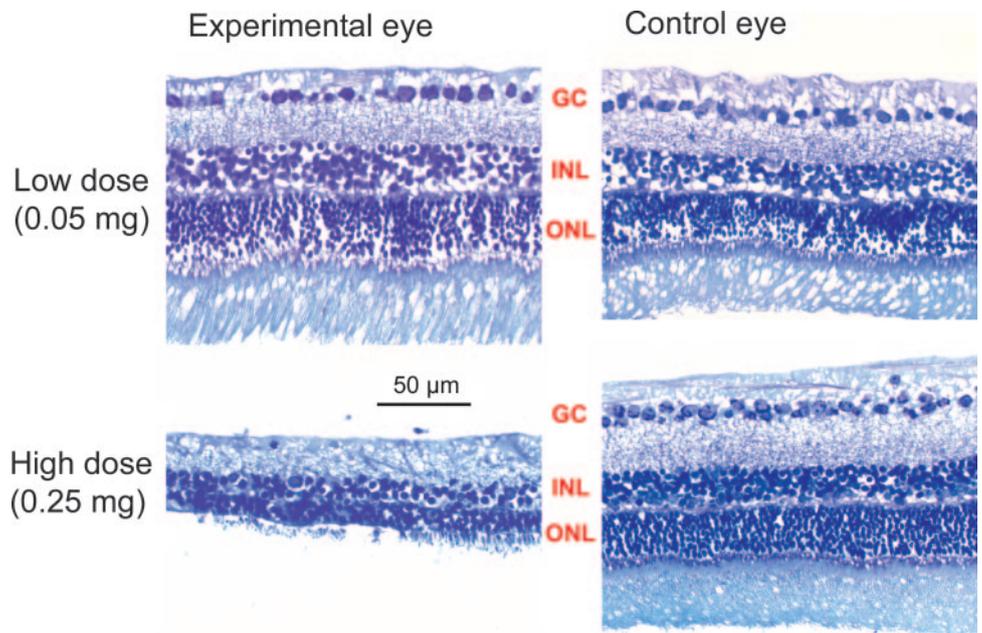

FIGURE 7. Histologic findings of experimental and control retinas from two rabbits, one treated with the highest dose (0.25 mg) of ICG (*bottom row*) and the other with the lowest (0.05 mg) dose (*top row*). Micrographs show areas close to the site of injection. The three layers of cell nuclei, ONL, INL, and GC, appear normal in the control retinas (*right column*) and in the experimental retina exposed to the lowest dose of ICG (*top left*). The retina that was exposed to the highest dose of ICG is thin and appeared severely damaged. Scale bar, 50 μm.

change. Because ICG caused ERG diminution, we conclude that ICG, diffusing through the retina from the vitreal side, can exert toxic actions on distal retinal neurons. ICG toxicity to the distal retina, including the RPE, has also been demonstrated by morphologic analysis of the rabbit retina after subretinal injection of ICG,[26,27] as well as in a porcine model of retinal hole.[25]

Findings in studies of donor human eyes[21] and of human RPE cells in culture[23,34] have suggested that ICG toxicity is intensified by illumination. In our experiments, however, the rabbits were exposed to bright light only during the intravitreal injection procedure, after which they were kept in darkness for 3 hours before the first-injection ERG recordings, which showed functional deficit (Fig. 4). Thus, photic damage can be excluded in the present study. Another mechanism of ICG toxicity was attributed to the hypo-osmolarity of the solvent.[35] This mechanism could not be involved in our model because a volume of 0.1 mL of ICG solution was injected into a vitreous of approximately 1.47 mL in volume (for rabbits weighing 1.8–2.7 kg),[36] leading to only a minor change in vitreal osmolarity. Furthermore, in control experiments, we tested the effects of 0.1 mL distilled water on retinal function, and found no ERG deficit for as long as 4 weeks of follow-up (Table 2). Thus, it is likely that several pathways are involved in ICG-induced neuronal damage.

The effects of ICG on the VEP indicate reduced retinal output to the brain and subnormal conduction via the optic nerve. These findings could reflect ICG-induced damage to distal retinal neurons, to proximal retinal neurons, or to both. Because ICG was found to be toxic to rat retinal ganglion cells,[30] we suggest that the drug is toxic to all retinal cells, leading to deterioration of the flash-elicited ERG and VEP responses.

The findings reported herein and by others[28,29] indicate that intravitreal injection of ICG in rabbits or rats can cause permanent structural and functional damage to the retina. The relevance of these observations to the use of ICG in vitreoretinal surgery should be considered. In the animal studies, ICG was injected into the vitreous and cleared within 10 days in rats[28] or within 4 to 6 weeks in rabbits. In clinical practice, ICG is applied to the ILM for 10 to 60 seconds and then washed away, followed by a full vitrectomy. Thus, the time of exposure to ICG differs between clinical use and animal experimentation. Several recent reports, however, have described long-lasting fluorescence of ICG emanating from the retina, even though it had been washed away. Giardella et al.[37] reported four cases in which persistent ICG fluorescence in the central macula was detected up to 8 months after surgery. Ashikari et al.[38] reported two cases in which examination with a scanning laser ophthalmoscope detected fluorescence from residual ICG up to 9 months after surgery, even though no ICG staining of the retina was visible on clinical examination. Tadayoni et al.[39] reported on 17 patients who underwent vitrectomy with the use of ICG and demonstrated persistent diffuse fluorescence of the fundus at 1 and 3 months after surgery, with only the optic disc remaining fluorescent at 6 months after surgery. Weinberger et al.[40] reported one case in which persistent fluorescence was demonstrated 6 weeks after vitrectomy, but there was normal function with improved visual acuity and regular amplitudes on multifocal ERG. These reports demonstrate long-lasting contact between ICG and retinal elements after use of ICG in vitreoretinal surgery raising the possibility of ICG toxicity.

Our experimental approach did not mimic the clinical use of ICG. However, taking our data together with the many other reports on ICG toxicity in animal models, cadaveric human eyes, and cultured human retinal cells, of ICG should be used in vitreoretinal surgery with caution. The concentration of the dye should be maintained at a minimal level that will still be

beneficial for the surgeon. The time of exposure should be as short as possible, and washout should be meticulous to prevent long-standing exposure of the retina to ICG.[29]

References

1. Destro M, Puliafito CA. Indocyanine green videoangiography of choroidal neovascularization. *Ophthalmology*. 1989;96:846–853.
2. Guyer DR, Puliafito CA, Mones JM, et al. Digital indocyanine green angiography in chorioretinal disorders. *Ophthalmology*. 1992;99:287–291.
3. Yannuzzi LA, Slakter JS, Sorenson JA, et al. Digital indocyanine green videoangiography and choroidal neovascularization. *Retina*. 1992;12:191–223.
4. Kadonosono K, Itoh E, Uchio E, Nakamura S, Ohno S. Staining of internal limiting membrane in macular hole surgery. *Arch Ophthalmol*. 2000;118:1116–1118.
5. Gandorfer A, Messmer EM, Ulbig MW, Kampik A. Indocyanine green selectively stains the internal limiting membrane. *Am J Ophthalmol*. 2001;131:387–388.
6. Da Mata AP, Burk SE, Reimann CD, et al. Indocyanine green-assisted peeling of the internal limiting membrane during vitrectomy surgery for macular hole repair. *Ophthalmology*. 2001;108:1187–1192.
7. Eshita T, Ishida S, Shinoda K, et al. Indocyanine green can distinguish posterior vitreous cortex from internal limiting membrane during vitrectomy with removal of epiretinal membrane. *Retina*. 2002;22:104–106.
8. Kumar A, Prakash G, Singh RP. Indocyanine green enhanced maculorhexis in macular hole surgery. *Indian J Ophthalmol*. 2002;50:123–126.
9. Kwok AK, Li WW, Pang CP, et al. Indocyanine green staining and removal of internal limiting membrane in macular hole surgery: histology and outcome. *Am J Ophthalmol*. 2001;132:178–183.
10. Burk SE, Da Mata AP, Snyder ME, Rosa RH Jr, Foster RE. Indocyanine green-assisted peeling of the retinal internal limiting membrane. *Ophthalmology*. 2000;107:2010–2014.
11. Haritoglou C, Neubauer AS, Gandorfer A, Thiel M, Kampik A. Indocyanine green for successful repair of a long standing macular hole. *Am J Ophthalmol*. 2003;136:389–391.
12. Haritoglou C, Gass CA, Schaumberger M, Ehrt O, Gandorfer A, Kampik A. Macular changes after peeling of the internal limiting membrane in macular hole surgery. *Am J Ophthalmol*. 2001;132:363–368.
13. Gass CA, Haritoglou C, Schaumberger M, Kampik A. Functional outcome of macular hole surgery with and without indocyanine green-assisted peeling of the internal limiting membrane. *Graefes Arch Clin Exp Ophthalmol*. 2003;241:716–720.
14. Haritoglou C, Gandorfer A, Gass CA, Schaumberger M, Ulbig MW, Kampik A. The effect of indocyanine green on functional outcome of macular pucker surgery. *Am J Ophthalmol*. 2003;135:328–337.
15. Uemura A, Kanda S, Sakamoto Y, Kita H. Visual field defects after uneventful vitrectomy for epiretinal membrane with indocyanine green-assisted internal limiting membrane peeling. *Am J Ophthalmol*. 2003;136:252–257.
16. Horio N, Horiguchi M. Effect on visual outcome after macular hole surgery when staining the internal limiting membrane with indocyanine green dye. *Arch Ophthalmol*. 2004;122:992–996.
17. Engelbrecht NE, Freeman J, Sternberg P Jr, et al. Retinal pigment epithelial changes after macular hole surgery with indocyanine green-assisted internal limiting membrane peeling. *Am J Ophthalmol*. 2002;133:89–94.
18. Haritoglou C, Gandorfer A, Gass CA, Schaumberger M, Ulbig MW, Kampik A. Indocyanine green-assisted peeling of the internal limiting membrane in macular hole surgery affects visual outcome: a clinicopathologic correlation. *Am J Ophthalmol*. 2002;134:836–841.
19. Sheidow TG, Blinder KJ, Holekamp N, et al. Outcome results in macular hole surgery: an evaluation of internal limiting membrane peeling with and without indocyanine green. *Ophthalmology*. 2003;110:1697–1701.

20. Gandorfer A, Haritoglou C, Gass CA, Ulbig MW, Kampik A. Indocyanine green-assisted peeling of the internal limiting membrane may cause retinal damage. *Am J Ophthalmol.* 2001;132:431–433.

21. Gandorfer A, Haritoglou C, Gandorfer A, Kampik A. Retinal damage from Indocyanine Green in experimental macular surgery. *Invest Ophthalmol Vis Sci.* 2003;44:316–323.

22. Ho JD, Tsai RJF, Chen SN, Chen HC. Cytotoxicity of indocyanine green on retinal pigment epithelium. *Arch Ophthalmol.* 2003;121:1423–1429.

23. Yam HF, Kwok AKH, Chan KP, et al. Effect of indocyanine green and illumination on gene expression in human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci.* 2003;44:370–377.

24. Grisanti S, Szurman P, Gelisken F, et al. Histological findings in experimental macular surgery with indocyanine green. *Invest Ophthalmol Vis Sci.* 2004;45:282–286.

25. Czajka MP, McCufen BW, Cummings TJ, Nguyen H, Stinnett S, Wong F. Effects of indocyanine green on the retina and retinal pigment epithelium in a porcine model of retinal hole. *Retina.* 2004;24:275–282.

26. Lee JE, Yoon TJ, Oun BS, Lee JS, Choi HY. Toxicity of Indocyanine green injected into the subretinal space. *Retina.* 2003;23:675–681.

27. Kawaji T, Hirata A, Inomata Y, Koga T, Tanihara H. Morphological damage in rabbit retina caused by subretinal injection of indocyanine green. *Graefes Arch Clin Exp Ophthalmol.* 2004;242:158–164.

28. Enaida H, Sakamoto T, Hisatomi T, Goto Y, Ishibashi T. Morphological and functional damage of the retina caused by intravitreous indocyanine green in rat eyes. *Graefes Arch Clin Exp Ophthalmol.* 2002;240:209–213.

29. Chao AN, Chen SN, Kuo YH. Retinal function and histologic changes following intravitreal injection of Indocyanine Green in a Rabbit Model. *J Ocul Pharmacol Ther.* 2004;20:450–459.

30. Iriyama A, Uchida S, Yanagi Y, et al. Effects of indocyanine green on retinal ganglion cells. *Invest Ophthalmol Vis Sci.* 2004;45:943–947.

31. Loewenstein A, Zemel E, Lazar M, Perlman I. Drug-induced retinal toxicity in albino rabbits: the effects of imipenem and aztreonam. *Invest Ophthalmol Vis Sci.* 1993;34:3466–3476.

32. Hood DC, Birch DG. A computational model of the amplitude and implicit time of the b-wave of the human ERG. *Vis Neurosci.* 1992;8:107–126.

33. Dong CJ, Hare WA. Contribution to the kinetics and amplitude of the electroretinogram b-wave by third-order retinal neurons in the rabbit retina. *Vision Res.* 2000;40:579–589.

34. Sippy BD, Engelbrecht NE, Hubbard GB, et al. Indocyanine green effect on cultured human retinal pigment epithelial cells: implication for macular hole surgery. *Am J Ophthalmol.* 2001;132:433–435.

35. Stalmans P, Van Aken EH, Veckeneer M, Feron EJ, Stalmans I. Toxic effect of indocyanine green on retinal pigment epithelium related to osmotic effects of the solvent. *Am J Ophthalmol.* 2002;134:282–285.

36. Prince JH. The vitreous. In: Prince JH, ed. *The Rabbit in Eye Research.* Springfield, IL: Charles C Thomas; 1964:372–384.

37. Giardella AP, Schiff W, Barile G, et al. Persistent indocyanine green fluorescence after vitrectomy for macular hole. *Am J Ophthalmol.* 2003;136:174–177.

38. Ashikari M, Ozeki H, Tomida K, Sakurai E, Tamai K, Ogura Y. Retention of dye after indocyanine green-assisted internal limiting membrane peeling. *Am J Ophthalmol.* 2003;136:172–174.

39. Tadayoni R, Paques M, Girmens JF, Massin P, Gaudric A. Persistence of fundus fluorescence after use of indocyanine green for macular surgery. *Ophthalmology.* 2003;110:604–608.

40. Weinberger AW, Kirchhof B, Mazainani BE, Schrage NF. Persistent indocyanine green (ICG) fluorescence 6 weeks after intraocular ICG administration for macular hole surgery. *Graefes Arch Clin Exp Ophthalmol.* 2001;239:388–390.