Kinetics of Indocyanine Green Dye After Intraocular Surgeries Using Indocyanine Green Staining

Masayuki Horiguchi, MD; Shima Nagata, MD; Naoki Yamamoto, PhD; Yoshihisa Kojima, MD; Yoshiaki Shimada, MD

Objective: To determine the elimination kinetics of indocyanine green (ICG) after intraocular operations using ICG staining.

Methods: Intraocular fluorescence of ICG was determined using the ICG angiographic mode of a scanning laser ophthalmoscope (in vivo) and fluorescence microscopy (in vitro) after circular curvilinear capsulorhexis with ICG staining during cataract surgery and internal limiting membrane (ILM) peeling with ICG staining during macular hole surgery.

Subjects: We studied 9 eyes of 7 patients with white cataracts and 14 eyes of 14 patients with idiopathic macular holes.

Results: Scanning laser ophthalmoscopy revealed fluorescence in the anterior segment of patients with cataracts on the first postoperative day, and fluorescence remained for a mean±SD of 6.0±2.2 days postoperatively. Scanning laser ophthalmoscopy also revealed fluorescence in the posterior pole of patients with macular holes, and it remained for a mean±SD of 2.7±1.4 months postoperatively. Fluorescence microscopy showed fluorescence of the entire tissues, suggesting that ICG had stained not only the surface of the membranes but had also entered them. In both operations, visual outcomes were not significantly different from the results obtained without ICG.

Conclusions: Because entire tissues were stained, the differences in ICG kinetics might also be caused by factors other than differences in stainability, such as the environment surrounding the tissues or molecular structural differences between the lens capsule and the ILM. Although we found complete disappearance of fluorescence and good functional recovery, the longer resident time of the dye after macular hole surgery may suggest a potential risk to intraocular tissues.

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In 1998, we described a technique of indocyanine green (ICG) staining for circular curvilinear capsulorhexis (CCC) in patients with mature white cataracts. This technique was also studied in enucleated human eyes and is now widely used throughout the world during cataract surgery. Recently, the ICG staining technique has also been used for peeling the internal limiting membrane (ILM) during macular surgery. This method facilitates difficult procedures by making the transparent membranes visible.

Because successful CCC in mature cataracts and ILM peeling during macular hole surgery are critical to anatomical and visual success, this staining technique is potentially beneficial for patients as well as surgeons. Although ICG has been used safely for examination of liver function, cardiac output, and choroidal circulation, how the dye affects the intraocular tissues has not been investigated extensively. Thus far, ICG staining has not caused serious problems in the early postoperative period, but a longer postoperative period must also be examined. To better understand the long-term effects on ocular tissues, it is important to understand the kinetics of ICG postoperatively, such as where and how long the dye stays in the eye. We studied the postoperative kinetics of ICG using the ICG angiographic mode of a scanning laser ophthalmoscope and a fluorescence microscope in patients with mature cataracts or macular holes.

Methods

Subjects

We studied 9 eyes of 7 patients with mature white cataracts and 14 eyes of 14 patients with idiopathic macular holes (Table 1 and Table 2). This study was performed in ad-
herence to the Declaration of Helsinki, and informed consent was obtained from each subject.

INDOCYANINE GREEN SOLUTION

In our original report, we used a 0.5% ICG solution, but recently we found that transparent tissues are still visible with lower concentrations of ICG solution. Therefore, we now use a 0.125% solution. The solution is made by injecting 2.0 mL of distilled water into a bottle with 25 mg of ICG powder (Daiichi Pharmaceutical Co, Tokyo, Japan) and shaking the bottle until the ICG is completely dissolved. Then, 1.5 mL of the dissolved ICG is aspirated from the bottle, and 4.5 mL of balanced salt solution plus is injected into the remaining 0.5 mL of ICG to make a final concentration of 0.125%. The osmolarity of the ICG solution was 270 mOsm, which is identical to that in our original report.

SURGICAL TECHNIQUES

Cataract Surgery

After a 3-mm corneal incision was made, the aqueous humor was replaced with viscoelastic material. Then, 0.1 to 0.2 mL of 0.125% ICG solution was injected under the viscoelastic material, and the dye spread on the capsule. The excess ICG and viscoelastic material were removed with an irrigation/aspiration tip, then the anterior chamber was refilled with viscoelastic material. This technique provided an excellent staining and view of the capsule, and CCC was performed successfully in all patients. The anterior segment tissues were exposed to ICG for approximately 10 to 30 seconds. Phacoemulsification and complete aspiration of the cortex were then performed, followed by intraocular lens implantation.

Macular Hole Surgery

A posterior vitreous detachment was formed with a cutter, and the vitreous was removed as much as possible. Without the infusion, 0.1 to 0.2 mL of 0.125% ICG solution was flushed on the surface of the retina, and the excess ICG in the vitreous cavity was removed by aspiration with a cutter. There was excellent visibility of the ILM, and it was successfully peeled off in all patients. The posterior segment tissues were exposed to ICG for approximately 10 to 30 seconds. Phacoemulsification and complete aspiration of the cortex were then performed, followed by intraocular lens implantation.

Table 1. Clinical Findings and Elimination Kinetics of Indocyanine Green in Patients With White Cataracts

<table>
<thead>
<tr>
<th>Patient No./ Age, y/Sex/Eye</th>
<th>Visual Acuity</th>
<th>Cell Count</th>
<th>Day of Fluorescence Disappearance†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preoperative</td>
<td>Postoperative</td>
<td>Preoperative, Count/mm²</td>
</tr>
<tr>
<td>1/64/M/R</td>
<td>HM</td>
<td>1.0</td>
<td>2932</td>
</tr>
<tr>
<td>2/79/F/L</td>
<td>HM</td>
<td>0.8</td>
<td>2657</td>
</tr>
<tr>
<td>3/78/F/R</td>
<td>0.02</td>
<td>0.7</td>
<td>2612</td>
</tr>
<tr>
<td>3/78/F/L</td>
<td>0.02</td>
<td>0.4</td>
<td>2638</td>
</tr>
<tr>
<td>4/28/F/R</td>
<td>HM</td>
<td>1.5</td>
<td>2625</td>
</tr>
<tr>
<td>4/28/F/L</td>
<td>HM</td>
<td>1.5</td>
<td>2309</td>
</tr>
<tr>
<td>5/76/M/R</td>
<td>0.05</td>
<td>0.9</td>
<td>2695</td>
</tr>
<tr>
<td>6/20/M/L</td>
<td>0.5</td>
<td>1.0</td>
<td>2463</td>
</tr>
<tr>
<td>7/79/F/R</td>
<td>LP</td>
<td>0.4</td>
<td>2371</td>
</tr>
</tbody>
</table>

Abbreviations: HM, hand motions at 30 cm; LP, light perception.
*The mean ± SD percentage loss was 6.5 ± 7.7.
†The number of days after the operation until fluorescence disappeared from the anterior capsule (mean ± SD, 6.0 ± 2.2 days).

Table 2. Clinical Findings and Elimination Kinetics of Indocyanine Green in Patients With Macular Holes

<table>
<thead>
<tr>
<th>Patient No./ Age, y/Sex/Eye</th>
<th>Visual Acuity</th>
<th>Last Tissue With Fluorescence†</th>
<th>Month of Fluorescence Disappearance‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preoperative</td>
<td>Postoperative</td>
<td>logMAR Improvement*</td>
</tr>
<tr>
<td>1/66/F/L</td>
<td>0.09</td>
<td>0.4</td>
<td>0.65</td>
</tr>
<tr>
<td>2/63/F/R</td>
<td>0.1</td>
<td>0.2</td>
<td>0.30</td>
</tr>
<tr>
<td>3/56/F/R</td>
<td>0.2</td>
<td>0.6</td>
<td>0.48</td>
</tr>
<tr>
<td>4/69/F/R</td>
<td>0.1</td>
<td>0.7</td>
<td>0.85</td>
</tr>
<tr>
<td>5/67/F/R</td>
<td>0.3</td>
<td>0.7</td>
<td>0.37</td>
</tr>
<tr>
<td>6/69/F/R</td>
<td>0.2</td>
<td>1.0</td>
<td>0.70</td>
</tr>
<tr>
<td>7/65/F/L</td>
<td>0.2</td>
<td>0.7</td>
<td>0.54</td>
</tr>
<tr>
<td>8/64/F/L</td>
<td>0.6</td>
<td>0.6</td>
<td>0.00</td>
</tr>
<tr>
<td>9/53/F/R</td>
<td>0.04</td>
<td>0.5</td>
<td>1.10</td>
</tr>
<tr>
<td>10/64/F/R</td>
<td>0.06</td>
<td>0.15</td>
<td>0.40</td>
</tr>
<tr>
<td>11/48/F/R</td>
<td>0.06</td>
<td>0.2</td>
<td>0.42</td>
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<tr>
<td>12/65/M/L</td>
<td>0.08</td>
<td>0.5</td>
<td>0.52</td>
</tr>
<tr>
<td>13/56/F/L</td>
<td>0.04</td>
<td>0.3</td>
<td>0.88</td>
</tr>
<tr>
<td>14/68/M/R</td>
<td>0.2</td>
<td>0.7</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Abbreviation: logMAR, logarithm of the minimum angle of resolution.
*The mean ± SD visual acuity improvement was 0.5 ± 0.28.
†The last region (ie, disc and/or fovea) in which fluorescence was observed by scanning laser ophthalmoscopy.
‡The postoperative month in which fundus fluorescence disappeared (mean ± SD, 2.7 ± 1.4 months).
for endoillumination was a 150-W halogen lamp, and no filter was used.

**OBSERVATION TECHNIQUES**

**In Vivo Analysis by Scanning Laser Ophthalmoscopy**

We examined the anterior and posterior segments of the eye using the ICG angiographic mode of an SLO. The SLO uses a 780-nm infrared light from a diode laser (2.0 mW) and allows the observation of the fluorescent ICG. Peak excitation of ICG is 805 nm, and the peak ICG emission is 835 nm. In patients who underwent surgery without ICG staining, no intrinsic fluorescence was observed in the posterior pole of the eye.

Postoperatively, we examined the anterior segment daily and the posterior segment monthly. In preliminary experiments, we found that a concentration of 0.1 ng/mm² of ICG was the detection threshold for our observation conditions. Thus, the absence of fluorescence does not necessarily mean that ICG was not present.

**In Vitro Analysis by Fluorescence Microscopy**

We modified a standard fluorescence microscope (BX60-F-SET; Olympus Optical Co, Tokyo, Japan) for observing the ICG in the excised lens capsule and ILM tissues. The light source was a 100-W halogen lamp, and a 710-nm to 820-nm excitor filter and a 800-nm to 950-nm barrier filter were used. The microscope was linked to a personal computer, and a detailed description of this microscope has been previously reported. In operations performed without ICG staining, the excised tissues were not fluorescent.

**RESULTS**

**CATARACT SURGERY**

All operations were completed without complication, and visual recovery was excellent. We found no remarkable loss of corneal endothelial cells and no excessive inflammation, as we reported previously. The loss of the endothelial cells was 8.09% on the first day postoperatively. All eyes showed fluorescence in the anterior segments on SLO (**Figure 1**). The edge of the capsule and the iris were fluorescent, and a small number of fluorescent cells were seen floating in the anterior chamber.

No fluorescence was found on corneal endothelial cells. The fluorescence disappeared within a mean±SD of 6.0±2.2 days postoperatively. The least number of days during which fluorescence was detected was 2, and the maximum was 9.

A photomicrograph of a cross-section of a lens capsule taken with the fluorescence microscope is shown in **Figure 2A**. Homogenous fluorescence was observed, indicating that the dye had stained not only the surface of the capsule but also the entire specimen. At higher magnification, the cytoplasm of the epithelial cells did not appear to be stained (Figure 2B).

**MACULAR HOLE SURGERY**

All operations were performed without complications, and the macular holes were closed, as was confirmed by optical coherence tomography. Improvement of logMAR (logarithm of the minimum angle of resolution) visual acuity in this study was not significantly different from that in 53 macular hole cases in our previous study, in which the ILM was removed without ICG staining (mean±SD: current study, 0.56±0.26 logMAR units; previous study, 0.48±0.26 logMAR units) (P=.26; Mann-Whitney U). No visual field defect was found by Goldmann perimetry.

Fluorescence was observed in the posterior pole for a mean±SD of 2.7±1.4 months postoperatively.
The area around the fluid cuff and macular hole, the optic disc, and the area with intact ILM were fluorescent. The least number of months during which fluorescence was detected was 1, and the maximum was 6. The time course for the disappearance of fluorescence varied for different tissues. Fluorescence from intact ILM disappeared first, and that of the disc and fovea persisted for a longer duration (Table 2).

A fluorescence photomicrograph of an ILM specimen is shown in Figure 4. Homogenous fluorescence of the tissue was observed, suggesting that ICG stained not only the surface of the ILM but also the entire specimen.

**COMMENT**

**KINETIC DIFFERENCES**

These results demonstrate that ICG can be detected in the anterior segment several days postoperatively when used for capsular staining during CCC and for several months postoperatively when used during ILM peeling. Although Weinberger et al reported persistent ICG fluorescence 6 weeks after macular hole surgery, the present study revealed longer persistence of the dye. Fluorescence microscopic examination of excised tissues revealed that the entire tissue was fluorescent, indicating that ICG dye had not only stained the surface but had also penetrated into the tissue. This implies that differences in the kinetics of ICG dye clearance are solely dependent on the staining properties of the tissues.

The anterior capsule is a basement membrane composed of type IV collagen and glycoproteins, and the ILM is also a basement membrane composed of type IV collagen and glycosaminoglycans. However, the proportions of these basement membrane constituents as well as variable concentrations of heparin sulfate, fibronectin, and proteoglycan; the presence of type I collagen in ILM; and the unique lectin binding properties of the lens capsule and ILM may play a significant role in the elimination kinetics of ICG.

The thickness of the anterior lens capsule differs from that of the ILM. The anterior capsule is about 20 µm thick and the ILM is 0.5 to 3.5 µm. However, this does not explain the difference in kinetics because the thinner ILM showed fluorescence for a longer duration.

Another difference between the 2 tissues is the in situ environment. In the anterior segment, aqueous humor is turned over at a flow rate of 3.5 µL/min, and both sides of the capsule are continually washed by the aqueous humor. In the vitreous cavity, the turnover rate of the fluid is unknown, and only the surface of the ILM is exposed to the vitreous. This may be one reason for the kinetic differences. In any case, persistence of the dye after macular hole surgery implies longer exposure of the tissues to ICG.

After macular hole surgery, ICG remained for a longer duration in the fovea and optic disc. It is possible that ICG penetrated beneath the retina and/or diffused into the retina through the macular hole during surgery and remained for a longer duration in the foveal region postoperatively. Because the ILM is thinnest at the optic disc, ICG might pass more easily into the nerve tissues through the ILM and remain in the region of the disc for a longer duration. The route of ICG clearance may vary depending on the site of tissue staining (ie, fovea, optic disc, or ILM).

**POTENTIAL TOXICITY OF RESIDUAL ICG**

**Cataract Surgery and Endothelium**

The ICG remaining in the eye can damage some intraocular tissues. In the anterior segment, ICG remained on the capsule, iris, and floating cells. The possible drainage route of ICG is the anterior chamber angle and the episcleral veins. Therefore, ICG will have contact with the corneal endothelial cells. In a previous study, we found no significant loss of endothelial cells in patients with cataracts without extremely hard nuclei. In this study, patients who retained fluorescence the longest showed only 8.1% endothelial cell loss. These observations suggest that the residual ICG did not adversely affect endothelial cells.

We did not find any changes in the iris or increased inflammation in the anterior segment. Consid-
ering the early disappearance of ICG fluorescence in the current study and our observations during at least the last 2 years, we conclude that ICG staining may be used safely in the anterior segment. Further, the experiments by Holly et al support our conclusion, showing that 3 minutes of exposure to 0.5% ICG does not affect endothelial cell integrity.

Visual Function After Macular Hole Surgery

No patients had visual field defects 6 months postoperatively. There has been no report to date of visual field loss owing to ICG staining of the ILM. However, ICG remained in the posterior segment for a mean ± SD of 2.7 ± 1.7 months postoperatively, and fluorescence microscopy revealed that the entire ILM stained, suggesting that the ICG dye had some contact with the axons of the ganglion cells. However, the absence of visual field defects does not necessarily indicate that a loss of nerve fibers has not occurred because loss of a small number of nerve fibers does not induce visual field defects. The receptive fields of the ganglion cells are large and overlapping, and the loss of some fibers can be compensated for by other fibers. Therefore, we cannot state definitively that ICG staining of the ILM is safe for nerve fibers. In addition, this technique was introduced very recently, and more observational and prospective studies are required before a definite conclusion can be made about its safety.

It is likely that ICG diffused between the detached retina and the retinal pigment epithelium during the operation. Although the ICG beneath the fovea may affect recovery of visual acuity, 15 of 16 patients had an improvement in visual acuity of more than 2 lines. In addition, patients with longer postoperative periods of fluorescence (patient 6, 6 months; patient 14, 5 months) showed an excellent recovery of visual acuity. The recovery of logMAR visual acuity in this study was not significantly different from that in our previous report, in which the ILM was peeled off without ICG staining. These results suggest that ICG did not adversely affect recovery of visual acuity in the short-term. However, Engelbrecht et al questioned the visual outcomes in patients with macular holes undergoing ICG-assisted ILM peeling. Further, some histological reports suggest that this dye is toxic to the tissues in the posterior pole. Careful longitudinal studies are required to make a final conclusion regarding the safety of ICG dye in posterior segment surgery.

CONCLUSIONS

The ICG molecules remain in the eye for some time postoperatively, and ICG stained not only the surface but also the entire tissue of the capsule and ILM. Because ICG remained for a longer duration in the eye after macular hole surgery, further investigations of the toxicity of ICG to retinal pigment epithelium and the retina are required.

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Corresponding author and reprints: Masayuki Horiguchi, MD, Department of Ophthalmology, Fujita Health University School of Medicine, Toyoake, Japan 470-1192 (e-mail: masayuki@fujita-hu.ac.jp).

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