Effect on Visual Outcome After Macular Hole Surgery When Staining the Internal Limiting Membrane With Indocyanine Green Dye

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Objectives: To determine the effect on the visual outcome after macular hole surgery when staining the internal limiting membrane (ILM) with indocyanine green (ICG) dye and to study the mechanism of the adverse effects.

Patients and Methods: We studied 40 eyes of 38 patients with an idiopathic macular hole (size, <0.5 disc diameter; duration, <12 months). The concentration, exposure time, and amount of the ICG solution that was minimally required to make the ILM visible were determined. The patients were randomly divided into group 1 (20 eyes of 19 patients) who underwent ILM peeling without ICG staining, and group 2 (20 eyes of 19 patients) who underwent ILM peeling with ICG staining. Routine examinations were conducted during the 12-month follow-up period. Multifocal electroretinogram, optical coherence tomography, and fluorescein angiography were performed on 31 eyes of 30 patients.

Results: The macular hole was closed in all patients. Visual acuity was improved in both groups, but it was significantly better in group 1 (median, 0.85) than in group 2 (median, 0.60; \( P = .02 \)) after 12 months. The improvement of visual acuity in group 1 (logarithm of the minimum angle of resolution [logMAR] units [SD], 0.82 [0.19]) was significantly better than that in group 2 (logMAR units, 0.67 [0.21]; \( P = .30 \)). The multifocal electroretinogram and optical coherence tomographic findings were not significantly different in the 2 groups. Fluorescein angiogram showed only weak hyperfluorescence at the macula in some patients of both groups.

Conclusions: The results suggest that ICG staining should not be used as long as the visibility of the retinal surface is good. However, ICG staining may be acceptable at a low concentration when a clear view of the retinal surface is unattainable. The results of the multifocal electroretinogram, optical coherence tomography, and fluorescein angiography suggest that the differences in visual recovery were caused not only by pigment epithelial cell damage or retinal toxic effect but also probably by the effect of ICG staining on ganglion cells and their axons.

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In 1998, we described a new surgical technique of staining the capsule of the crystalline lens with indocyanine green (ICG) dye during mature cataract surgery (ICG with continuous circular capsulorhexis [ICG-CCC]). Later, ICG staining was applied to the internal limiting membrane (ILM) to make it more visible during macular hole surgery (ICG-ILMhexis). Although our report on ICG-CCC was a randomized control study, to our knowledge, ICG-ILMhexis has not been studied in such a manner. In addition, the concentration of ICG solution in ICG-CCC was determined from the results of animal experiments; however, to our knowledge, the optimal concentration for human ICG-ILMhexis has not been determined.

Recently, we reported that the ICG dye remained in the eye for a long period after ICG-ILMhexis. This is important because there have been reports that ICG dye is a toxic substance and other groups have described the efficacy and safety of ICG-ILMhexis. Furthermore, in vitro studies using human retinal pigment epithelial (RPE) cells have demonstrated the toxic effect of ICG dye and illumination, an osmotic effect of the vehicle of ICG solution, and an ICG-induced decrease of mitochondrial enzyme activity. We have performed a randomized control study to determine the effect of ICG-ILMhexis on the visual outcome after macular hole surgery and to study the mechanism of the adverse effects.

METHODS

PATIENTS

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studied prospectively. Eyes with an old macular hole (>12 months), eyes with a large macular hole (>0.5 disc diameter), or eyes with atrophic RPE changes at the macular hole were excluded from the study. After obtaining informed consent, the study eyes were randomly divided before surgery into 2 groups using a coin-tossing method.

There were 20 eyes of 19 patients that underwent ILM peeling without ICG staining in group 1, and 20 eyes of 19 patients that underwent ILM peeling with ICG staining in group 2. Preoperatively, there was no significant difference in the visual acuity (range, group 1: 0.03-0.3; median, 0.125; group 2: 0.03-0.3; median, 0.15), the size and stage of the macular hole, the duration of the symptoms, and age of the patient (mean [SD] age, group 1: 63.5 [6.9] years; group 2: 64.7 [6.9] years; Table 1). The stage of the macular hole was determined during surgery.

**ICG SOLUTION**

We use a 0.125% solution of ICG dye. The solution was made by injecting 2.0 mL of sterile distilled water into a bottle of 25 mg of ICG dye (Daiichi Pharmaceutical Co Ltd, Tokyo, Japan, identical to Acorn product), and the bottle was shaken until the powdered ICG was completely dissolved. Then, 1.5 mL of the dissolved ICG was aspirated and 4.5 mL of an irrigating solution (BBS Plus; Alcon Inc, Forth Worth, Tex) was injected into the remaining 0.5 mL of ICG in the bottle to make a final concentration of 0.125%. The osmolarity of the ICG solution in the bottle was 270 mOsm that is identical to that reported in our original article.1

**SURGICAL TECHNIQUES**

A posterior vitreous detachment was formed with a cutter unless it was already present, and as much of the vitreous was removed as possible. The infusion of fluid was stopped, and 0.2 mL of the 0.125% ICG solution was flushed onto the surface of the retina. The ICG dye in the vitreous cavity was removed immediately by aspiration with the cutter.

With an excellent view of the ILM, the ILM was successfully peeled in all patients. Fluid-gas exchange was performed, and the vitreous was replaced by 12% perfluoropropane. The light source of endoillumination was a 150-W halogen lamp; no filter was used.

**CONCENTRATION, EXPOSURE TIME, AND AMOUNT OF ICG SOLUTION**

Before beginning this study, we determined the concentration, exposure time, and amount of ICG solution that was minimally needed to make the ILM visible during surgery (minimal staining). We found that 0.2 mL of a 0.125% ICG solution placed on the retina for 10 to 30 seconds was effective. The 0.125% ICG solution was diluted immediately after injection by the residual vitreous; the volume in the vitreous cavity varied among the patients. Therefore, the concentration of ICG solution in the vitreous cavity was not constant and was estimated to be 0.05 to 0.06 mg/mL (0.005%-0.006% with an assumed volume of the vitreous cavity of 4-5 mL). This concentration is much lower than the original report using ICG mixed with a viscomaterial.2 However, the concentration of the dye in the vitreous may differ from the concentration achieved at the vitreoretinal interface. The injected 0.125% ICG solution was aspirated immediately, but the exposure time of the ICG solution on the retina, the time from the start of the injection to the end of aspiration, was not constant, and was estimated to be 10 to 30 seconds from a review of the videotape taken during the surgery.

### Table 1. Patient Data

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of eyes/No. of patients</td>
<td>20/19</td>
<td>20/19</td>
<td>NA</td>
</tr>
<tr>
<td>Male/female</td>
<td>5/15</td>
<td>7/12</td>
<td>.48</td>
</tr>
<tr>
<td>Age, y</td>
<td>63.50 (6.9)</td>
<td>64.70 (6.9)</td>
<td>.57</td>
</tr>
<tr>
<td>Eye, R/L</td>
<td>14/6</td>
<td>11/9</td>
<td>.51</td>
</tr>
<tr>
<td>Preoperative visual acuity, range (mean[SD]), logMAR units</td>
<td>0.03-0.30</td>
<td>0.03-0.30</td>
<td>.87</td>
</tr>
<tr>
<td>Stage (mean[SD])</td>
<td>(0.92 [0.24])</td>
<td>(0.92 [0.25])</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>10</td>
<td>.43</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Size, disc diameter</td>
<td>0.22 (0.12)</td>
<td>0.27 (0.15)</td>
<td>.37</td>
</tr>
<tr>
<td>Axial length, mm</td>
<td>23.7 (1.3)</td>
<td>23.1 (1.1)</td>
<td>.10</td>
</tr>
<tr>
<td>Duration from symptom to surgery, mo</td>
<td>4.1 (4.2)</td>
<td>3.8 (3.1)</td>
<td>.99</td>
</tr>
<tr>
<td>Follow-up period, mo</td>
<td>17.0 (4.5)</td>
<td>18.7 (5.9)</td>
<td>.33</td>
</tr>
</tbody>
</table>

Abbreviations: logMar, logarithm of the minimum angle of resolution; NA, not applicable.

*Data are given as mean [SD] unless otherwise indicated.

**VISUAL ACUITY**

The visual acuity was measured preoperatively and postoperatively. We have reported that the preoperative visual acuity in patients with a macular hole is greatly affected by the patient's fixation and the use of a multiple letter chart can solve the problem.15 Unfortunately, the multiple letter chart was unavailable at the start of this study; therefore, we used a standard visual acuity chart (EA-117D; Takada Co Ltd, Tokyo Japan) that is widely used in Japan.

**MULTIFOCAL ELECTRORETINOGRAMS**

Multifocal stimulation and analyses were performed using the VERIS Science 4.1 system (Electro-Diagnostic Imaging, San Mateo, Calif, and Meiyo, Aichi, Japan). Multifocal electroretinograms (mERGs) were recorded from 16 eyes of 15 patients in group 1 and 15 eyes of 15 patients in group 2 from whom informed consent was obtained.

A stimulus array of 61 densely packed hexagons covered the central 50°. Within each frame of the cathode ray tube, each stimulus hexagon was either flashed on at an intensity of 2.67 candela (cd)/s per square meter or remained dark (<0.01 cd·s·m −2) and modulated at an m-sequence (215−1 steps).

The mERGs were recorded with a Burian-Allen bipolar contact lens electrode, amplified (×50000), and bandpass filtered between 10 and 300 Hz. K1 and K2 were extracted using the fast m-transform algorithm, and we measured the implicit times and the amplitudes of the largest peaks of the first-order kernel (b wave) of the central hexagon that fell on the fovea. Because the amplitudes of the mERG are highly variable, the ratio of the amplitudes, affected eye to fellow eye (A/F) ratio, was used to compare the 2 groups.16

**OPTICAL COHERENCE TOMOGRAPHY**

Cross-sectional images of the retina were obtained by optical coherence tomography (OCT) (Humphrey Instruments, San Leandro, Calif). The measurement of the foveal thickness was made manually using the longitudinal reflectivity profile in a scan profile program of the OCT.17,18 For the measurement, cursors were placed at the steepest part of each rising slope produced at the ILM and RPE. The retinal thickness used for analysis was

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the average of 4 measurements at the fovea. Optical coherence tomography was performed on 16 eyes of 15 patients in group 1 and 15 eyes of 15 patients in group 2.

OTHER MEASUREMENTS

Fluorescein angiography (FA), ophthalmoscopy, and Goldmann perimetry were performed during the follow-up period.

STATISTICAL ANALYSIS

Data are shown as the mean (SD). Statistical analysis software (SigmaStat; Jandel Scientific, San Rafael, Calif) was used for statistical comparisons. Comparisons for the 2 groups were performed using unpaired t test, and the Mann-Whitney test was performed for comparisons of visual acuity. Values of P<.05 were considered to be statistically significant.

RESULTS

VISUAL ACUITY

The median visual acuity in both groups was the same at 0.60 at 6 months after surgery, but at 12 months, the median visual acuity in group 1 was significantly better at 0.85 than in group 2 at 0.60 (P=.02; Figure 1). The improvements of visual acuity in logMAR (logarithm of the minimum angle of resolution) units are shown in Figure 2 for both groups. At 12 months, the improvements were significantly better in group 1 (0.82 [0.19]) than in group 2 (0.67 [0.21], P=.03).

A comparison of the preoperative and postoperative visual acuities at 12 months in all eyes is shown in Figure 3. All eyes had an improvement of visual acuity of more than 2 lines on the letter chart. The results of visual acuity in the 31 eyes of 30 patients who had mfERGs, OCT, and FA tests were consistent. Although group 2 included 5 more patients with a stage 4 macular hole (Table 1), their postoperative visual acuity ranged from 0.3 to 1.0 (median, 0.6) and so was not the cause of the difference in postoperative visual acuity.

MULTIFOCAL ELECTRORETINOGRAMS

There was no significant difference in the implicit time or the A/F ratio of the b-wave amplitudes preoperatively. The ratio of the amplitudes and peak time of the b wave of the mfERGs from the central hexagon were not significantly different for the 2 groups postoperatively (Table 2).
OPTICAL COHERENCE TOMOGRAPHY

In all cases, OCT showed a complete closure of the macular hole, and the thickness of the retina in the center of the fovea was not significantly different in the 2 groups (mean [SD], group 1: 147.9 [29.7] μm; group 2: 169.6 [58.2] μm; Table 2).

OTHER EXAMINATIONS

Fluorescein angiography showed only a faint hyperfluorescence in the macula in 7 of 16 eyes in group 1 and 8 of 13 eyes in group 2. Dilated fundus examination revealed no obvious atrophy of the RPE. Goldmann perimetry revealed no scotoma or constriction of the peripheral field.

Table 2. Multifocal Electroretinogram and Foveal Thickness*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b-Wave amplitude, affected/fellow eye</td>
<td>0.42 (0.16)</td>
<td>0.49 (0.10)</td>
<td>.38</td>
</tr>
<tr>
<td>b-Wave implicit time, ms</td>
<td>28.40 (1.80)</td>
<td>28.00 (1.60)</td>
<td>.70</td>
</tr>
<tr>
<td>Postoperative data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b-Wave amplitude, affected/fellow eye</td>
<td>0.83 (0.15)</td>
<td>0.78 (0.21)</td>
<td>.44</td>
</tr>
<tr>
<td>b-Wave implicit time, ms</td>
<td>28.40 (1.30)</td>
<td>28.40 (1.60)</td>
<td>.86</td>
</tr>
<tr>
<td>Foveal thickness, μm</td>
<td>147.90 (29.70)</td>
<td>169.60 (58.20)</td>
<td>.20</td>
</tr>
</tbody>
</table>

*Data are given as mean (SD).

COMMENT

MILD DEPRESSED RECOVERY OF VISUAL ACUITY BY ICG-ILMrhexis

Our data showed that the visual acuity and the improvement of visual acuity in the 2 groups were not significantly different 6 months after surgery. However, at 12 months, the recovery of the visual acuity was significantly better in eyes that were not stained with ICG than in eyes with ICG-ILMrhexis. This long-term effect is probably due to the delayed clearance of the dye after surgery because we have found that the dye remained in the eye for a mean (SD) of 2.7 (1.4) months after macular surgery in contrast to the rapid clearance after ICG-CCC (6.0 [2.2] days). Both groups achieved good recovery of visual acuity. This suggests that ICG toxicity did exist but is limited in this study.

Our results indicated that ICG staining might be acceptable at a low concentration in selected cases. For example, in an eye with media opacity that prevents a clear view of the retinal surface, ICG staining may be used to identify the ILM, but ICG staining should be avoided when the retinal surface is clearly visible.

MECHANISM OF THE DELAY IN RECOVERY OF VISUAL ACUITY

The mechanism of the effect of ICG staining on the visual acuity at 12 months has not been determined. The mfERGs were not significantly different in the 2 groups suggesting that the retinal cells giving rise to the mfERGs (photoreceptors, bipolar cells, and Muller cells) were minimally affected by ICG staining. The OCT-determined foveal thicknesses were also not significantly different in the 2 groups suggesting that there was no retinal edema or atrophy at the fovea. Fluorescein angiography showed only faint hyperfluorescence in some cases in both groups suggesting that RPE atrophy and retinal damage were not obvious as described previously in both groups.

Thus, these data suggest that the possible mechanism of the depressed visual acuity in the eyes exposed to ICG dye is damage to the axons of the ganglion cells or the nerve fibers at the optic nerve head. Recent reports stated that ICG dye remains at the optic nerve head for a long time after ICG-ILMrhexis. The findings from an experimental report demonstrating ICG dye in retinal ganglion cells after intravitreal injection support this possibility. Furthermore, photodynamic effects may account for the nerve fiber layer and ganglion cell damage. However, there may have been subclinical changes at the center of the fovea that could not be detected by mfERG, OCT, or FA. Several in vitro experiments have shown the toxic effect of ICG dye on the human RPE. We do not have a clinical test for measuring the function of RPE in the fovea. Therefore, we cannot exclude the possibility that dysfunction of foveal RPE affected visual acuity. To make a more definitive conclusion, further examinations of the visual fields and the RPE with longer follow-up periods are needed.

CONCENTRATION, EXPOSURE TIME, AND AMOUNT OF ICG SOLUTION

The use of ICG-ILMrhexis has been controversial. While some investigators reported poor results, other investigators reported good results. One of the reasons for this confusion is that the optimal concentration, exposure time, and quantity of ICG solution have not been determined. In our study, we determined a combination of the concentration, exposure time, and amount of ICG solution for minimal staining, which may be different among the hospitals because of the difference in instruments, source of the ICG dye, and color of the fundus in different races/ethnicities. Our results indicated that even a minimal staining procedure affects the visual acuity slowly and slightly; thus, we recommend that ICG dye might be used only when the retinal surface is not clearly visible.

CONCLUSIONS

Visual recovery after macular hole surgery was affected by ICG-ILMrhexis, even when it was performed using a minimal staining technique. However, because the effect was slight, ICG staining may be acceptable at a low concentration for a short time in eyes with media opacity that prevents a clear view of the retinal surface.
Both authors contributed equally to the work and therefore should be regarded as equivalent senior authors.

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REFERENCES


