Histological Findings in Experimental Macular Surgery with Indocyanine Green

Salvatore Grisanti,1 Peter Szurman,1 Falk Gelisken,1 Sabin Aisenbrey,2 Jolanta Oficjalska-Mlynczak,3 and Karl Ulrich Bartz-Schmidt1

PURPOSE. To analyze the effect of different concentrations and application intervals of indocyanine green (ICG) on the retina in an experimental setting of macular surgery.

METHODS. Twenty-one porcine eyes were used within 5 hours after enucleation. The eyes were hemisectioned and the vitreous removed. Different doses of ICG (up to 1 mg) were applied over the trephined macula, and the remainder of the eyecup was filled with a balanced salt solution (BSS). Both the ICG solution and the BSS were drained after 30 or 60 seconds and the complete eyecup irrigated and filled with fresh BSS. The posterior pole was then illuminated with a standard surgical light pipe and light source at maximum power for 3 minutes. Both the ICG-treated retina and the nontreated surrounding retina were processed for histology.

RESULTS. Exposure of the retina at different concentrations of ICG for 30 or 60 seconds, followed by illumination, caused no histologically detectable damage compared with the controls. No microarchitectural disorganization or cellular disruption was detected. The vitreoretinal interface seemed unaffected.

CONCLUSIONS. Previously described severe damage to the inner retina of human donor eyes could not be found with even higher doses of ICG in this porcine model. Although differences within the species may contribute to these contradictory results, it is conceivable that the postmortem time and the vitality of the tissue influence the outcome in this ex vivo system.

T he inner limiting membrane (ILM) is a physiological structure at the vitreoretinal interface. Though many functions have been attributed to this basal lamina, intentional removal of the ILM has become a routine procedure in macular hole surgery. Because of the poor visibility of the ILM, however, complete removal is difficult and not always obvious. Therefore, damage at the vitreoretinal interface or unsatisfactory outcomes may be the consequence of this surgical maneuver.

From the 1Center of Ophthalmology, Department of Vitreoretinal Surgery, Eberhard-Karls University Tübingen, Tübingen, Germany; the 2Department of Neuroscience, Tufts University, Boston, Massachusetts; and the 3Department of Ophthalmology, University of Wroclaw, Wroclaw, Poland.

Supported by the Vitreoret Foundation.

Submitted for publication July 28, 2003; accepted September 13, 2003.

Disclosure: S. Grisanti, None; P. Szurman, None; F. Gelisken, None; S. Aisenbrey, None; J. Oficjalska-Mlynczak, None; K. Ulrich Bartz-Schmidt, None.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked ‘advertisements’ in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Salvatore Grisanti, Center of Ophthalmology, Department of Vitreoretinal Surgery, Eberhard-Karls University Tübingen, Schleichstrasse 12-15, 72076, Tübingen, Germany; salvatore.grisanti@med.uni-tuebingen.de.

Since the first report by Grizzard and Tornambe1 and publications by Kadonosono et al.2 and Burk et al.3 application of indocyanine green (ICG) became a useful tool in facilitating ILM peeling.4–15

With gaining experience, however, some clinical studies reported concerns about a possible toxic effect of ICG on the neurosensory retina and the RPE.14–16 To examine whether ICG is toxic or not some laboratory studies have been performed and published.17–21

Gandorfer et al.20 recently developed an elegant experimental model of macular surgery to study the assumed toxic and photodynamic effect of ICG at the vitreoretinal interface. In their study, the authors processed human donor eyes enucleated 16 to 30 hours after death. After 0.05 mL of 0.05% ICG were applied on the macula for 1 minute, and the dye was drained by irrigation, the treated macula was illuminated for 3 minutes with a standard light source and by filtering different wavelengths for 3 minutes. In the authors’ opinion this experimental setting reflects the situation in clinics. Gandorfer et al. demonstrated that retinal specimens subjected to this process experienced severe damage, including loss of the ILM, cellular disorganization, and fragmentation of the cytoplasm.

Inspired by this study, we repeated the experiments to answer the following questions: Does ICG have the same effect on fresher tissue? Is there a safe ICG dose that can be used? Is the effect based on osmolarity or pH?

To answer these questions and to mimic the clinical situation better, the experimental setting had to be modified. Because our results are contradictory to the above-mentioned study, the clinical importance of both experimental settings will be discussed critically.

MATERIALS AND METHODS

Tissue Preparation

Twenty-one adult porcine eyes were received from the local abattoir. The freshly enucleated porcine eyes were transported to the laboratory in ice and used within 5 hours after death. The eyes were processed by using a modification of the method described by Gandorfer et al.20 After removal of the anterior segment, we additionally removed the vitreous. A trephine of 9-mm diameter was positioned over the posterior pole afterward and left in place to create a chamber that allowed a standardized application of ICG dye (Pulsion, Munich, Germany) without treating the areas outside the chamber. The eyecup itself created a second chamber surrounding the trephine. This second chamber was filled with balanced salt solution (BSS; Pharmacia, Groningen, The Netherlands) as a control (Fig. 1A).

A 25-mg vial of ICG dye was reconstituted with distilled water. The concentration of this ICG stock solution was 10 mg/mL. The stock solution was further diluted with BSS to attain the experimental concentrations 0.1, 1, and 2 mg/mL of ICG. Because the volume used within the trephine chamber was 0.5 mL, the absolute doses of ICG applied to the retina were 1, 0.5, and 0.05 mg, respectively. The ICG solutions were poured into the trephine and left in contact with the retina for 30 seconds or 1 minute, respectively. The removal of the dye

Copyright © Association for Research in Vision and Ophthalmology

282
was followed by the removal of the trephine and irrigation of the eyecup with fresh BSS.

In consequence, the posterior pole was illuminated with a new standard light pipe (Spectra-Band Fiberoptic Endo-Illuminator; Altomed Ltd., Tyne and Wear, UK) and the fiberoptic light source (50 W; Pentalux; Ruck, Eschweiler, Germany) set to a maximum illumination power. According to Gandorfer et al.\(^2\) the fiberoptic was placed 8 mm above the posterior pole. The illuminated area included both the ICG-stained area and part of the surrounding control area (Fig. 2B). Thereafter, retinal specimens from the illuminated untreated area and from the illuminated ICG-treated area were obtained. The specimens were placed in phosphate-buffered 4% glutaraldehyde solution for fixation. postfixed with Dalton fixative (osmium 2%), dehydrated, and embedded in Epon. Semithin sections were stained with toluidine blue for light microscopy.

Each experiment was repeated three times. The experimental groups are shown in Table 1. Six groups were characterized by three different doses of ICG (1, 0.5, and 0.05 mg) and two different incubation times (30 seconds and 1 minute). The seventh group was used as an additional control to detect the influence of the hypo-osmolar stock solution (distilled water) without ICG. The osmolarity and pH of the experimental and control solution were measured, and the values are

---

**Figure 1.** Experimental ex vivo model of macular surgery. (A) A trephine is positioned over the posterior pole and filled with ICG solution. The remainder of the eyecup is filled with BSS. (B) After the removal of the ICG solution and the trephine the eyecup is rinsed with fresh BSS, and the fiberoptic illuminates both the stained ICG-treated (✽) and the untreated areas. Specimens from both areas (white boxes) were examined by histology.

**Figure 2.** Light microphotographs of toluidine blue-stained semithin sections of treated (A, C) and control (B, D) retinal specimens, each taken from the same eye. (A) Specimen from group 2 incubated with 1 mg ICG for 60 seconds followed by illumination. (B) Specimen from group 6 incubated with 0.05 mg ICG for 60 seconds and then illuminated. All specimens showed different degrees of vacuolization, but had well-preserved cytoarchitecture, and the ILM remained attached.
and not to the ICG.

showed that the decrease was attributable solely to the solvent (distilled water) in the final solution. The osmolarity correlated with the amount of solvent (distilled water) in the final solution. In group 7, the distilled water without ICG was used instead of the stock solution.

In groups 1 to 6, ICG was applied at different concentrations for 30 or 60 seconds, respectively. The ICG stock solution (distilled water) influenced the osmolarity of the final solution. In group 7, the distilled water without ICG was used instead of the stock solution.

<table>
<thead>
<tr>
<th>Eyes (n = 3)</th>
<th>Volume from Stock Solution (mL)</th>
<th>ICG Concentration (mg/mL)</th>
<th>ICG Dose (mg)</th>
<th>Time (sec)</th>
<th>pH</th>
<th>OsM/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.100</td>
<td>2.0</td>
<td>1.00</td>
<td>30</td>
<td>7.15</td>
<td>0.248</td>
</tr>
<tr>
<td>2</td>
<td>0.100</td>
<td>2.0</td>
<td>1.00</td>
<td>60</td>
<td>7.15</td>
<td>0.248</td>
</tr>
<tr>
<td>3</td>
<td>0.050</td>
<td>1.0</td>
<td>0.50</td>
<td>30</td>
<td>7.17</td>
<td>0.271</td>
</tr>
<tr>
<td>4</td>
<td>0.050</td>
<td>1.0</td>
<td>0.50</td>
<td>60</td>
<td>7.17</td>
<td>0.271</td>
</tr>
<tr>
<td>5</td>
<td>0.005</td>
<td>0.1</td>
<td>0.05</td>
<td>30</td>
<td>7.17</td>
<td>0.306</td>
</tr>
<tr>
<td>6</td>
<td>0.005</td>
<td>0.1</td>
<td>0.05</td>
<td>60</td>
<td>7.17</td>
<td>0.306</td>
</tr>
<tr>
<td>7</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>60</td>
<td>7.15</td>
<td>0.248</td>
</tr>
</tbody>
</table>

In groups 1 to 6, ICG was applied at different concentrations for 30 or 60 seconds, respectively. The ICG stock solution (distilled water) influenced the osmolarity of the final solution. In group 7, the distilled water without ICG was used instead of the stock solution.

Representative semithin sections of the treated and untreated specimens of group 2 (highest ICG dose) and group 6 (lowest ICG dose) are shown in Figure 1. All the specimens had a well-preserved retinal cytoarchitecture. This result was consistent in all 42 specimens that were examined. A common finding in all groups and in both the treated and untreated areas was a spongiform vacuolization of the retina. The specimens were evaluated by two observers independently and in a blind fashion, to see whether more severe vacuolization occurred in a special group, but this was not the case. Furthermore, in all sections no disruption of the nerve fibers or of the ganglion cells was detected. No disorganization of the cytoarchitecture, as previously described, was found in any of the specimens.

The ILM in porcine eyes is much thinner than in human eyes and almost unrecognizable by light microscopy. However, analysis by electron microscopy disclosed that the ILM was attached both in the experimental and control groups. Ultrastructural analysis also demonstrated intact cells, cell membranes, and cellular organelles. There was no difference between treated and untreated eyes. Measurements of the pH of the applied solutions showed that neither the concentration of ICG nor the ICG solvent (distilled water) had an influence.

ICG also had no detectable influence on the osmolarity of the solution. The osmolarity correlated with the amount of solvent (distilled water) in the final solution. Increasing amounts of distilled water from the stock solution lead, as expected, to a decrease in osmolarity down to 0.248 OsM/kg. Measurement of the osmolarity of the solution in group 7 showed that the decrease was attributable solely to the solvent and not to the ICG.

**DISCUSSION**

The tricarbocyanine dye ICG was introduced into ophthalmology by Flower and Hochheimer in 1973 to study the choroidal circulation. The hydrophilic dye binds strongly to proteins, but is excluded from living cells by intact cell membranes. The tendency of ICG to bind to basement membranes was first recognized by cataract surgeons and used to improve visualization of the anterior lens capsule to facilitate performing the anterior capsulorrhexis in dense white cataract. It was then also noticed by vitrectinal surgeons, who introduced the dye in macular hole surgery to improve identification and to facilitate complete removal of the ILM from the posterior pole.

The enthusiasm associated with this controlled and improved technique of ILM-peeling led to widespread use of ICG, and several publications have reported experiences with the staining of the ILM. Though most of the reports presented positive results with good functional outcome, some other reports have contributed to the growing suspicion that ICG may be toxic to the RPE and the neurosensory retina.

Engelbrecht et al. published a clinical series that found central RPE atrophy with poor visual outcomes after some cases of ICG-assisted macular hole repair. The defect was thought to be a consequence of direct contact of ICG with RPE cells in the area of the hole. Haritoglou et al. reported less improvement of visual acuity and more visual field defects in ICG-assisted macular hole surgery. The histologic examination of the peeled membranes disclosed cellular elements, leading to the assumption that ICG may cause retinal damage by altering the cleavage plane. However, a histopathological study of the ILM-peeling without ICG disclosed a similar accumulation of cellular.

Because the clinical reports were contradictory, concerns about retinal and RPE toxicity led to laboratory studies, including in vitro, in vivo, and ex vivo experiments.

What have we learned from the posterior-segment–related in vitro studies that were performed exclusively with RPE cells? Sippy et al. demonstrated that human RPE cells, incubated for 20 minutes with a 0.05% ICG solution and illuminated for 10 minutes, did not show any histologic or ultrastructural changes compared with the control. However, cells that were subjected to these conditions showed a significant decrease in enzymatic activity. Stalmans et al. reported that human RPE in culture exposed for 5 minutes to an ICG solution (concentration not shown) in fact were severely affected. They also demonstrated that this effect was not based on ICG itself, but on the hypotonic (247 mOsm/kg) solution. ICG contains iodine to enhance its solubility and must be dissolved in pure water, before bringing it to its final concentration.

Though in vitro studies can be performed in a controlled fashion, in vivo studies are needed to mimic the clinical situation better. So far, there is only one published study examining the effect of ICG on the retina in vivo. Enaida et al. chose a rat model. Two weeks after vitrectomy by injection of 0.05 mL SF6 gas, the authors injected (0.05 mL/eye) different concentrations of ICG (0.025–25 mg/mL), reflecting a dose of 0.00125 to 1.25 mg per eye. The ICG solution was left in the eye. Retinal toxicity was assessed by light microscopy and electrophotography. The study showed that after 10 days, the eyes that were injected with an ICG dose of 0.125 mg and higher
had a severely affected retina. Though no apparent histopathologic change was observed in the low-dose (0.0125 mg/eye) groups, even these eyes showed functional damage on ERG. These results are to be taken into serious account, because the doses used are within or below the range (0.05–5.0 mg/eye) of clinical application. However, several factors exclude the clinical relevance of this article. First, the dose–tissue relationships are not comparable. A dose of 0.125 mg injected into a rat eye with a volume of 0.5 cm³ is expected to have a higher impact than the same dose injected into an eight-times larger human eye with a volume of 4 cm³. Second, in clinical practice, the ICG solution is removed within seconds or minutes, and only a minimal amount of residual ICG would have an extended effect.

An important contribution has also been made by Gandorfer et al., who documented in their ex vivo study the effect ICG and illumination applied in a fashion similar to that used in surgical practice. They could demonstrate that exposure of the retina to ICG and illumination of a certain wavelength would induce severe damage to the cytoarchitecture at the vitreoretinal interface.

Because a disastrous outcome like this would have led to the loss of reading ability in all our patients treated with ICG-assisted peeling, we questioned whether these results may be related to the long postmortem interval. Gandorfer et al. argued that the effects were solely related to the ICG and the illumination. They supported their opinion by comparing the histology of treated and nontreated retina of the same eye. However, an appropriate control would have been to treat the control retina in the same way except for the application of ICG. This means that the control retina should have been exposed to a non-ICG solution, containing the same amount of the solvent (hypo-osmolar) and should have been irrigated at the end of the incubation. But in the study, the control remained completely untouched and protected by the vitreous except for the illumination.

To examine whether fresher tissue reacts differently, we were interested in whether there is a safe ICG dose that can be used, and whether the reported effect was based on osmolarity or pH of the ICG solution. We repeated the experiment. To better mimic the clinical situation we had to modify the experimental setting. Though we used by far higher doses of ICG than Gandorfer et al., in our study we could not see the changes described by them. There was no disorganization of the retinal cytoarchitecture and no cellular disruption. There was no histologic or ultrastructural difference between experimental and control specimen. There was no ICG dose or osmolarity-related change. We therefore had to ask ourselves why there is a discrepancy between our results and those of Gandorfer et al.

One reason could be that we used porcine eyes. The rationale for using porcine eyes was: (a) a greater number of comparable eyes to reduce the number of variables; and (b) reducing the time between death and the experiments.

Because the porcine retina is similar to the human retina, we do not believe that our results are significantly based on the difference between these two species. We believe that the contradictory results between Gandorfer et al. and our study are based on the following points:

1. Gandorfer et al. report that their donor eyes were enucleated 16 to 30 hours after death. We do not know how much time then passed between enucleation and the experiments. Our porcine eyes in contrast were processed within 5 hours after death.
2. After death and enucleation, our porcine eyes were placed on ice until the experiments were performed. This reduces the progression of cellular damage and autolysis.
3. In the study by Gandorfer et al. the vitreous was not removed. This does not reflect the situation during surgery. With the vitreous in place, a different degree of accumulation at the vitreoretinal interface may have occurred.
4. The adjacent retina that was used as a control by Gandorfer et al. was completely untouched, except for the illumination.

After death, tissue undergoes a process of devitalization that of course leads to autolysis with time. Without being already autolytic, tissue in ex vivo experiments is more sensitive to any kind of affection. The more devitalized the cells, the more reduced is their ability to react properly and to resist sources of degradation (e.g., free radicals). Furthermore, photodynamic effects, on one hand, are primarily based on an intracellular reaction; ICG, on the other hand, usually does not penetrate a healthy cell membrane easily. It is conceivable that postmortem damage of the cell membrane allows the accumulation of intracellular ICG and the exertion of a photodynamic effect, as described by Gandorfer et al. Therefore, the interpretation of ex vivo experiments should be scrutinized very critically. In our opinion, our results are closer to the effect of ICG in vivo. However, appropriate in vivo experiments should be conducted to demonstrate this.

We believe that there is no question that ICG, and other dyes as well, may be toxic and may also be phototoxic to the exposed tissues. However, the level of toxicity depends on the dose and the incubation time. We should not forget that the solvent may have negative effects too. Therefore, abuse of ICG or other dyes should be avoided. The purpose of using dyes is not to provide a beautifully stained ILM, but a barely more visible membrane to make surgery more controlled, safer, and faster. This is also a way to reduce phototoxicity to the macula. Experienced surgeons may not need and should therefore not use ILM staining. However, less experienced surgeons within their learning curve may cause much less damage to the retina by using a dye within a safe limit than without. Therefore, a general condemnation of ICG or other vital dyes is probably wrong as is uncritical acceptance. As is shown by the most recent reports, increased experience with vital staining of the ILM is leading to a titration of the dose and the exposure time used.

References


