Histology of the Vitreoretinal Interface after Indocyanine Green Staining of the ILM, with Illumination Using a Halogen and Xenon Light Source

Christos Haritoglou, Siegfried Priglinger, Arnd Gandorfer, Ulrich Welge-Lussen, and Anselm Kampik

PURPOSE. To assess the histology of the retinal surface after staining of the inner limiting membrane (ILM) with indocyanine green (ICG) followed by illumination with halogen or xenon light sources in human donor and porcine eyes.

METHODS. Ten eyes of six human donors and six porcine eyes were used in the study. In human donor eyes, the postmortem time varied between 7 and 38 hours, and porcine eyes were evaluated 9 hours after death. In all eyes, the vitreous was removed, and a few drops of 0.5% ICG were poured over the trephined posterior pole and carefully washed out after a period of 1 minute, with balanced salt solution. Then the stained retina was illuminated for 3 minutes with different light sources: a halogen light source of 145-W power or a xenon light source of 50-W power. Adjacent, unstained retina of each eye served as a control to assess postmortem artifacts. In two human and two porcine eyes ICG was applied without illumination. Retinal specimens were evaluated by light and electron microscopy.

RESULTS. In human eyes, severe disorganization of the innermost retina and ILM loss were observed after ICG application with subsequent illumination with the halogen light source. After illumination with the xenon light source, there was only slight vacuolization of the innermost retina, with mostly intact Müller cells. The ILM remained in situ in relation to the retinal surface. Intact cellular architecture was found in all specimens after ICG staining without subsequent illumination and control specimens of unstained retina. In porcine eyes, no impact attributable to the light source or ICG alone was noted in this experimental setting.

CONCLUSIONS. These findings suggest that adverse effects of ICG at the retinal surface may depend on the light source used during vitrectomy and correlate with the emission spectrum of the different light sources. In addition, care should be taken when comparing results obtained in human eyes and porcine eyes. (Invest Ophthalmol Vis Sci. 2005;46:1468–1472) DOI: 10.1167/iovs.04-0838

Removal of the internal limiting membrane (ILM) has been shown to be beneficial for the repair of traction maculopathies, such as macular holes and macular pucker.1-3 However, detection and removal of the ILM represents a challenging procedure for the vitreoretinal surgeon. The introduction of indocyanine green (ICG) to stain the ILM4 was met with great enthusiasm, as the ILM could be stained selectively,5 and the removal of this delicate structure was greatly facilitated. After initial reports on ICG staining6,7 functional results after ICG-assisted vitrectomy were contradictory, with some groups describing unfavorable functional results8-11 and others observing no dye-related problems.12,13 Besides other experimental studies indicating ICG-related adverse effects,14-17 it was suggested that a photosensitizing effect might be induced at the retinal surface by an overlap of the emission spectrum of the light source and the absorption band of the ICG solution resulting in morphologic damage at the retinal surface.18 It was also shown that the light-absorbing properties of an ICG solution depend on the solute, with maximum absorption between wavelengths of 600 and 700 nm when one of two saline solutions (BSS or BSS Plus; Alcon, Fort Worth, TX) were used for dilution.19 The morphologic alterations of the inner retina18 were observed when the stained retinal surface was illuminated with a commonly available halogen light source emitting light between 380 and 760 nm, with a maximum at approximately 560 nm. The emission of xenon light sources is different from that of halogen light sources, with a shift of the maximum spectral radiance toward a lower wavelength of approximately 450 nm.20 In theory, this may limit the overlap between the emission spectrum of the light source and the absorption band of ICG. As a consequence, considering the photosensitizing properties of ICG, morphologic alterations may be less pronounced or completely absent when the stained retinal surface is illuminated with a xenon light source. The following study was performed to evaluate this hypothesis. In addition, we wanted to elucidate reported differences21 in the outcome of morphologic evaluations of the vitreoretinal interface in an ex vivo animal model using porcine eyes, by comparing the postmortem morphology of the stained and unstained vitreoretinal interface of human and porcine eyes.

MATERIALS AND METHODS

The impact of different light sources on the histology of the vitreoretinal interface after ICG staining with subsequent illumination was evaluated in 10 eyes of six human donors and in 6 porcine eyes. The human eyes were obtained within 7 to 38 hours after death after informed consent was obtained for experimental studies, in accordance with the guidelines for the Declaration of Helsinki for research involving human tissue. None of the donors had a relevant general medical or ophthalmic history that would interfere with the experiments planned or would have any impact on the interpretation. The cornea was conserved for transplantation. The age of the donors ranged from 18 to 68 years (Table 1). Studies in porcine eyes were performed 9 hours after death. In all eyes, the experimental setting was as follows: After removal of the lens and the iris (Fig. 1A), a 12-mm trephine was slowly moved through the vitreous and the posterior pole. With the trephine kept in place, tissue surrounding the trephined posterior pole was carefully removed. These specimens of untreated...
was used in six human donor eyes (and two porcine eyes). In two (and two porcine eyes) in this study, whereas the xenon light source was performed in only two human donor eyes.

The table further indicates which treatment was applied in each eye and summarizes the histological findings. Unstained and nonilluminated retina was taken from each eye and served as a control, as did specimens of unstained retina with illumination from each light source alone. These controls are not listed in the table. Two eyes were treated with ICG only, two with ICG staining and subsequent illumination with the halogen light source, and six with ICG staining and subsequent illumination with the xenon light source.

retina of all eyes were conserved as a control to assess possible postmortem artifacts and autolysis and were also used to assess the impact of illumination on the unstained retina alone. Then, the vitreous was carefully removed from the trephined posterior pole and 2 to 3 drops of 0.5% ICG (Pulsion, Munich, Germany) were poured into the trephine (Fig. 1B) and washed out after a period of 1 minute (BSS Plus; Alcon Laboratories), followed by illumination of the stained retinas (Fig. 1C) for 3 minutes with two different light sources: a halogen light source of 145-W power (Geuder, Heidelberg, Germany) or a 50-W xenon light source (Pentalux; Ruck, Eschweiler, Germany). As during surgery, the fiberoptic was placed approximately 8 mm over the stained retinal surface of human donor eyes had been described by the authors in a similar setting,18 illumination with the halogen light source on the stained retinal surface of human donor eyes had been performed in six grids containing at least three ultra-thin sections of each specimen, by another examiner (CH), with the treatment unmasked.

FIGURE 1. Globe and specimen preparation. First, the lens and the iris were removed (A). Then a 12-mm trephine was slowly moved through the vitreous and the posterior pole. With the trephine kept in place, the tissue surrounding the trephined posterior pole was carefully removed. These specimens of untreated retina of all eyes served as a control, to assess possible postmortem artifacts and autolysis. The vitreous was carefully removed from the trephined posterior pole, and 2 to 3 drops of 0.5% ICG were poured within the trephine (B), which served as an artificial chamber. After a period of 1 minute, the dye was carefully washed out with saline solution, followed by illumination of the stained retina (C) for 3 minutes with a halogen or xenon light source. Then the trephine was removed and the tissue was prepared for histologic evaluation.

### Table 1. Age and Postmortem Times of Each Donor

<table>
<thead>
<tr>
<th>Human Donor Age and Postmortem Time</th>
<th>Treatment</th>
<th>Histological Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>45 years/38 hours 68 years/15 hours</td>
<td>One eye of each donor: ICG + halogen light (145 W)</td>
<td>Gross disorganization of inner retina, ILM absent, and Müller cell endfeet and processes damaged.</td>
</tr>
</tbody>
</table>
| 18 years/10 hours 65 years/37 hours | Eye 1: ICG + xenon light (50 W)  
Eye 2: ICG, no illumination | Eye 1: ILM present, Müller cell endfeet and processes intact, empty sub-ILM spaces, and focal ILM detachment.  
Eye 2: ILM present, with unremarkable cytoarchitecture. |
| 65 years/24 hours 51 years/7 hours | Eye 1 and 2: ICG + xenon light (50 W) | ILM present, Müller cell endfeet and processes intact, empty sub-ILM spaces, and focal ILM detachment. |

### Results

#### Human Donor Eyes

**Halogen Light Source.** In semithin sections, we found a marked disorganization of the inner retinal layers. The ILM was absent in all specimens (Fig. 2A). These findings were observed in all eyes and were independent of postmortem time and donor age. Control specimens (Fig. 2B) and specimens after ICG staining without further illumination (Fig. 2C) showed a smooth retinal surface, with the ILM being in situ in all sections, similar to specimens after halogen light illumination of the unstained retina. Vacuolization within the retina as a sign of autolysis was noted frequently.

Electron microscopy of stained specimens revealed fragmentation of the cytoplasm and cellular organelles (Fig. 3A) at the level of the inner retina, as well as disintegrated Müller cell processes and end feed in all sections. In contrast, the cytoarchitecture of the retina appeared well preserved in all specimens that received ICG with no additional illumination. Similar observations were made in control specimens without ICG staining (Fig. 3B). There were mild postmortem changes present in all specimens.

**Xenon Light Source.** When the xenon light source was used for illumination, the ILM was present providing a smooth retinal surface both in specimens of a donor eye of a young donor (18 years) with a short postmortem time of 10 hours (Fig. 4A) and in specimens of an elderly donor (65 years) with prolonged postmortem time of >37 hours (Fig. 4B). Vacuoles and empty spaces had formed under the ILM (Figs. 4A, 4B) in all specimens, and, in some sections, an additional focal detachment of the ILM (Fig. 4B) was noted. Signs of autolysis were more pronounced in the eye with prolonged postmortem time of 37 hours (Fig. 4B). In all control specimens taken from...
the same eye as shown in Fig. A, we observed vacuolization as a sign of autolysis but no ILM detachment (Fig. 4C). Similar observations were made in all specimens of unstained retina with xenon light illumination alone.

Under higher magnification with electron microscopy, the ILM was present in all specimens (Figs. 5A, 5B). Most Müller cell processes and end feed appeared intact and electron dense (Fig. 5A) although some damaged Müller cells could be seen in close proximity (Fig. 5B). Empty spaces were detected adjacent to affected or unaffected Müller cell processes (Figs. 5A, 5B) containing cellular debris possibly originating from damaged Müller cell end feed. Control specimens (Fig. 5C) revealed well-preserved tissue with intact Müller cells as did the specimen treated with ICG only (Fig. 5D).

Porcine Eyes: Halogen and Xenon Light Sources

In contrast to the morphologic alterations in human donor eyes, no such findings were made in porcine eyes, with the same experimental approach. As in control specimens of unstained retina without illumination (Fig. 6D), semithin sections of retinal specimens provided a smooth surface with well preserved cellular architecture after ICG staining alone, as well as after ICG staining followed by illumination with both light sources (Figs. 6A–C).

**DISCUSSION**

**Human Donor Eyes**

In the present postmortem study in human donor eyes, we found differences regarding the ultrastructure of the retinal surface, depending on the light source used for illumination after ICG staining: Although there were no obvious abnormalities in specimens after application of ICG alone (without subsequent illumination) or in control specimens of unstained retina, damage to the inner retinal layers after ICG staining and illumination was noted with use of the halogen light source (145 W), as reported previously. In contrast, different observations were made when using a xenon light source (50 W), with ultrastructural abnormalities being much less pronounced. The histologic findings after ICG staining and illumination with the 50-W xenon light source were confirmed both in a donor eye of a young donor with short postmortem time and in an older donor with prolonged postmortem time, which may indicate the validity of the results in terms of postmortem tissue vulnerability and autolytic changes.

With respect to the photosensitizing properties of ICG, a possible explanation of our observations may be the different spectral irradiance of halogen and xenon light sources. The halogen light source used in our experiments has been reported to emit light between 380 and 760 nm with a maximum at 550 nm. Others have reported emissions between 380 and 760 nm.
700 nm, with a maximum at approximately 650 nm. In contrast, the spectral irradiance of a xenon light was reported to range between 350 and 700 nm, with a maximum at approximately 460 nm. The light-absorbing properties of ICG have been shown to depend on the solute and concentration, with a maximum absorption beyond 600 nm and practically no absorption at lower wavelengths of ~500 nm. As a consequence, the overlap of the emission of the light source and the absorption spectrum of ICG can be limited by choosing a xenon light source for illumination. This may reduce the risk of a photosensitizing effect at the retinal surface.

Porcine Eyes

Of note, the morphologic findings observed in our experimental setting in human eyes were not confirmed in porcine eyes using the same experimental approach. However, our results in porcine eyes are in line with the observations of other...
investigators who recently found no damage to the inner retinal layers in a porcine model, with the same xenon light source as was used in our experiments.\textsuperscript{21} This difference may be due to variations of the anatomy of the human and porcine eye. A comparative study has described variations of the vitreoretinal border within mammals, showing that the morphology of the ILM in primates differs markedly from that in nonprimates, by revealing complex folds in the equatorial and macular region and by its thickness, most pronounced in the macular region.\textsuperscript{22} Those investigators therefore concluded that nonprimate animal models should be considered less suitable when studying the vitreoretinal border and associated diseases. As a consequence, a transfer of results obtained from studies in porcine eyes to the human eyes appears difficult, at least with respect to the staining properties of the macula.

In summary, we are aware of the limitations and weaknesses of morphologic evaluations performed in postmortem tissue attributed to postmortem time and vitality of the tissue. Retinal anatomy and physiology are difficult to study after death, especially in regard to toxicity. The presented ex vivo approach does not completely mimic the intraoperative in vivo situation when performing ICG-assisted ILM peeling. In addition, the sample size was rather small, because of limited access to human donor tissue. Nevertheless, the results of such morphologic evaluations can provide useful information on the interactions of ICG illumination at the level of the retinal surface. Morphologic studies as presented may serve as a reference for further investigations in vivo.

References