Quantification of Contrast Recognizability during Brilliant Blue G– and Indocyanine Green–Assisted Chromovitrectomy

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PURPOSE. To evaluate the potential of brilliant blue G (BBG) and indocyanine green (ICG) for intraoperative staining of the internal limiting membrane (ILM) with respect to perceivable contrast.

METHODS. In a retrospective clinical case series the authors analyzed 26 consecutive chromovitrectomy interventions in 26 patients with macular holes, epiretinal fibrosis, vitreoretinal traction syndromes, or persistent macular edema. Fourteen subjects underwent ICG and 12 subjects, BBG chromovitrectomy. The main outcome measure was the difference in chromaticity between the stained ILM and the unstained underlying retina, measured by means of a novel objective and quantitative video-based analysis method to describe color contrast strengths as they are perceived by the human eye.

RESULTS. Objective chromaticity measurements of the intraoperative videos of all 26 interventions showed a significantly inferior contrast for BBG compared with that of ICG (BBG = 6.1, ICG = 14.9; P = 3.885 × 10⁻¹⁵).

CONCLUSIONS. As an adjunct to chromovitrectomy to stain the ILM, BBG yields a significantly less well discernible contrast to the human eye than that of ICG under the premises of this study. (Invest Ophthalmol Vis Sci. 2011;52:4345–4349) DOI: 10.1167/iovs.10-6972

The use of vital dyes during vitreoretinal surgery with the intention of improving the safety and ease of removing the internal limiting membrane (ILM), frequently referred to as chromovitrectomy,¹ has encountered growing and worldwide acceptance within the vitreoretinal community in recent years. Indocyanine green (ICG) has been used for almost a decade for this intention of improving the safety and ease of removing the internal limiting membrane (ILM), frequently referred to as chromovitrectomy,¹ has encountered growing and worldwide acceptance within the vitreoretinal community in recent years. Indocyanine green (ICG) has been used for almost a decade for this purpose²; however, although its staining characteristics are undisputed, a controversy over the clinical relevance of numerous reports on ICG toxicity continues to divide the experts.³–⁷ ICG is approved for intravenous use, whereas its intravitreal application represents an off-label use. With the introduction of brilliant blue G (BBG; Brilliant Peel; Geuder AG, Heidelberg, Germany), an alternative substance became available. BBG is approved for intravitreal use in European Union countries and, although in vitro toxicity has recently been reported,⁸ initial clinical reports demonstrate favorable functional results with no apparent toxicity.⁹–¹¹ Although both vital dyes selectively stain the ILM, staining properties have been reported to be weaker for BBG than those for ICG in subjective surgeon rating scores.¹¹ To elucidate the more gradual than expected acceptance of BBG as the standard of care in chromovitrectomy, we sought to quantify and compare the contrast visible to the human eye created by both substances.

MATERIAL AND METHODS

In a retrospective, multicenter, nonrandomized clinical case series, we analyzed 26 consecutive chromovitrectomy interventions in 26 patients with macular holes (MHs), epiretinal fibrosis (ERF), persistent macular edema (PME), and/or vitreoretinal traction syndrome (VRTS). Fourteen subjects underwent ICG and 12 subjects BBG chromovitrectomy (Table 1). Thirteen right eyes and 13 left eyes were included. Patient age ranged from 49 to 90 years (median, 76 years). Eleven patients were women; 15 patients were men. Exclusion criteria included patient under 18 years of age, technically poor video quality, previous chromovitrectomies within the preceding 6 months, and the use of additional vital dyes other than BBG or ICG during the same intervention, including the application of trypan blue to visualize epiretinal material.

All patients underwent routine 23-gauge vitrectomy, performed by three surgeons (PBH, SP, TJ) at two centers (Linz, Austria; Basel, Switzerland) using a vitrectomy system (OS 3; Oertli, Berneck, Switzerland) in combination with a light source (Photon II; Synergetics, O’Fallon, MO). Two patients were phakic, 17 were pseudophakic, and in 7 cases, cataract surgery was performed at the beginning of a combined operation (Table 1).

The decision to use either BBG or ICG was based on the preference of the individual surgeon. The sequential use of both BBG and ICG was permitted according to the surgical routine of both study centers, in which case, however, only the first dye was evaluated to exclude the effects of possible dye interactions. After complete posterior vitreous detachment, either BBG from ready-to-use 0.5-mL vials (Brilliant Peel) was injected into the vitreous cavity at a concentration of 0.25 mg/mL, followed by immediate clearance as recommended in the package leaflet, or an iso-osmol solution of ICG was instilled at a concentration of 1.25 mg/mL, with washout occurring after 60 seconds (ICG Pulsion; Pulsion Medical Systems AG, Munich, Germany), following the clinical routine of both institutions.

Membrane removal was recorded using a digital camera (Medlife Trio; Carl Zeiss Meditec, Jena, Germany) in connection with a digital recorder (Medlife Mind Stream; Carl Zeiss Meditec) attached to an
As part of the recording system setup routine, exposure and calibration alignments were performed, adjusting the white balance of the recording system to the white dropper of a standardized balancing screen (XpoBalance; Lastolight Ltd., Coalville, Leicestershire, UK) at the beginning of each intervention.

For quantitative analysis, the video sequences were viewed and analyzed postoperatively using a custom-made software tool programmed in MATLAB (version R2007b), a high-level language and interactive programming environment for scientific computing. The objective of this operation was to quantify the color contrast as it is perceived by the human eye. Frames displaying good image quality and maximum staining within the vascular arcades were selected and regions of interest (ROIs) representing maximum contrast were signaled by a vitreoretinal surgeon (PBH). Two distinct methods were used: in the single-image method one ROI was selected in an area with maximally stained ILM and compared with another adjacent ROI of similar dimensions in the same image in an area where the ILM had already been removed during the course of the procedure. In the multiple-image method, an area with maximum staining was selected and the same ROI was assessed at different points in time, before and after ILM removal. In total, 52 measurements with the single-image method and 81 measurements using the multiple-image method were performed (Figs. 1 and 2).

To quantitatively compare the perceived color contrast between selected ROIs, a methodology was used\(^\text{12}\) that had first been described by MacAdam in 1942, based on his systematic empiric analyses of human visual sensitivities to color differences. Within the CIE 1931 (where CIE stands for Commission internationale de l’eclairage [French for International Commission on Illumination]) chromaticity diagram, a classical vector space, where lights having the same color are represented as a point,\(^\text{13}\) MacAdam had defined regions containing all colors indistinguishable from the color located at the center of the region to the average human eye (Fig. 3, left). These regions feature an ellipsoid shape and are now known as MacAdam ellipses. The number of ellipses located between two distinct colors in the chromaticity diagram is a direct measure for their visually perceived color contrast. Because the regions vary in size and orientation depending on their center color, this metric was perceived as inexpedient and an attempt to create a less distorted representation led to development of the CIELAB (CIE 1976 \(L^*, a^*, b^*\)) color space, in which colors appear according to their discriminability by the human eye. A transcription of Table 1. Overview of Patient Sample

<table>
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<th>Patient</th>
<th>Vital Dye</th>
<th>Age (y)</th>
<th>Eye</th>
<th>Pathology</th>
<th>Sex</th>
<th>Lens Status</th>
<th>CIELAB Contrast Score</th>
<th>Measurements (n)</th>
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<td>Combined operation</td>
<td>10.2</td>
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<td>7.4</td>
<td>6</td>
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</tbody>
</table>

CIELAB, CIE 1976 \(L^*, a^*, b^*\) color space; OS, oculus sinister (left eye); OD, oculus dexter (right eye).

...
MacAdam ellipses to the CIELAB color space results in an almost, although not exact, circular distribution of indistinguishable colors (Fig. 3, right).

To assess the perceived color contrast between two selected ROIs, the custom-made software application first calculated the average color of all the pixels within each of the two ROIs. The two averaged colors were then projected into the CIELAB color space. This color space is perceptually uniform and changes of the same visual importance are reflected in an identical distance within the color space (Euclidean distance). The Euclidean distance can thus be regarded as a direct measure for the strength of perceived contrast. To ensure invariance to different lighting, caused for example by the vignetting artifact, the Euclidean distance was calculated only over the chromaticity components \( a^* \) and \( b^* \), neglecting the lightness \( L^* \).

To statistically analyze the significance levels of our measurements, we used the software package R (version 2.11.1, provided in the public domain by R Foundation for Statistical Computing, Vienna, Austria, available at http://www.r-project.org). Normality of the distributions was tested with the Kolmogorov–Smirnov test and all our measurements were normally distributed. A value of \( P < 0.05 \) was considered statistically significant for all \( t \)-tests in this study.

At all times, the tenets of the Declaration of Helsinki were observed. The protocol was approved by the local Institutional Human Experimentation Committee.

**RESULTS**

A clinically useful staining was observed for both BBG and ICG in most applications: in 25 patients, staining with the dye of first choice was sufficient for ILM removal, whereas in one intervention originally carried out with BBG, additional ICG was needed (patient 19, Table 1).

Chromaticity diagram analyses were performed for all 26 patients. CIELAB distances between the stained and the unstained retinas were significantly greater in the 14 videos from the ICG group than those in the 12 videos from the BBG group, representing a stronger contrast for ICG compared with that of BBG, both based on single-image (ICG = 14.7, BBG = 6.80, \( P = 7 \times 10^{-5} \)) and multiple-image (ICG = 15.0, BBG = 5.6, \( P = 4.0 \times 10^{-12} \)) measurements (Fig. 4).

A \( t \)-test showed no statistical difference between the single- and the multiple-image measurement methods (\( P = 0.86 \) for ICG; \( P = 0.29 \) for BBG), allowing pooling of data. Average CIELAB distances were 6.1 for BBG and 14.9 for ICG (\( P = 3.885 \times 10^{-15} \)).

**DISCUSSION**

The causative role of anteroposterior and tangential posterior pole vitreoretinal traction in the formation of a wide range of macular conditions, including VRTS, MH, ERF, and PME, is well documented. Common clinical features of these diseases include visual loss and metamorphopsias.

A fundamental approach to disorders caused by vitreoretinal traction has become available with the advent of pars plana vitrectomy. Mechanical vitrectomy tends to be incomplete, however, and remnants of the cortical vitreous have been demonstrated to remain adherent to the ILM, subject to proliferation of cells and continued traction. Although initially described as an inadvertent byproduct of the removal of
epiretinal membranes, peeling of the ILM is now advocated as a prudent adjunct to conventional vitrectomy in the eyes of patients with advanced macular vitreoretinal interface disorders. The transparency and tenuousness of the ILM certainly make its surgical removal technically challenging. Injury to the underlying retinal layers may occur, resulting in intraretinal hemorrhages, central retinal breaks, and functional retinal damage. To improve intraoperative visibility of the target tissue, the tricarbocyanine dye ICG has been proposed for selective intraoperative staining of the ILM. Although its intravitreal application constitutes an off-label use, it has become a standard adjunct for ILM peeling in recent years. ICG intravitreal application constitutes an off-label use, it has been implicated to cause toxic retinal and optic nerve damage, with less favorable visual acuity outcomes and visual field defects.

The triphenylmethane BBG has recently been introduced as an alternative vital dye for chromovitrectomy. It is available as a ready-to-use sterile solution approved for intraocular use in the European Union (Brilliant Peel). BBG selectively stains the ILM within a few seconds. Although a recent in vitro study elicited dose- and time-dependent BBG cell toxicity in a human retinal pigment epithelial cell line (ARPE-19), BBG has shown good outcomes and no apparent toxicity in initial clinical studies. Despite these obvious advantages over ICG, the acceptance of BBG in the retinal surgical community has been occurring rather sluggishly and ICG continues to be the most satisfactory staining capability to both ICG and BBG: after immediate in our patient sample, as recommended in the manufacturer’s instruction leaflet. The effect of variations in clinical application protocols on contrast strength has yet to be evaluated, but improved staining is expected with higher concentrations and extended exposure times based on preclinical findings.

Chromaticity measurements may similarly be influenced by the irradiation emission spectrum of the light source. All interventions of the present study were carried out using a mercury vapor light source (Photon II), characterized by a relatively narrow near green light spectrum and a minimal overlap with BBG and ICG absorption spectra, so that results do not necessarily reflect circumstances for the use of lighting equipment with broader emission spectra. With respect to the lens status, chromaticity scores may also have been somewhat influenced by the presence of a natural versus an artificial lens, due to a difference in pigment composition and, thus, different light-filtering effects. Only 2 of 26 patients were phakic, however. Both phakic patients belonged to the ICG group and both their average CIELAB score values were within the 25th to 75th percentiles, so that the effect appears to be negligible, although statistical proof cannot be provided due to small sample size.

We believe that staining comportment is the most important determinant of intraoperative utility of vital dyes. Notwithstanding the limitations of this study, and although ILM removal is principally possible with BBG in most cases, clearly inferior ILM staining properties for BBG compared with those for ICG could well contribute to the slower than expected
acceptance BBG is experiencing for chromovitrectomy, despite its obvious advantages over ICG with respect to approval status and toxicity profile.

Further studies with larger sample sizes should examine whether factors such as patient age, underlying pathologies, lens status, dye concentrations, exposure times, application with or without fluid-air exchange, and lighting devices influence staining compartment. Other aspects of vital dye utility, such as a presumed ICG facilitation of ILM removal through an alteration of ILM material properties, could represent an additional rationale for BBG's decelerated promulgation and also deserve further future inquiry. Ultimately, the objective of future research should be to contribute to the development of new application protocols for existing vital dyes or the introduction of new substances, which would combine the satisfactory staining characteristics of ICG with the favorable toxicity profile of BBG.

CONCLUSIONS

Intravitreal BBG provides a significantly less well discernible contrast between the ILM and the unstained retina to the human eye than intravitreal ICG under the premises of this study. The comparatively weak contrast provided by BBG may respond, at least in part, for the relatively slow acceptance of BBG as the standard of care in chromovitrectomy, despite its obvious advantages with regard to approval status and toxicity profile.

References