

Anatomical and functional outcome in brilliant blue G assisted chromovitrectomy

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ABSTRACT.

Purpose: To evaluate the potential of brilliant blue G (BBG) for intraoperative staining of the inner limiting membrane (ILM) with respect to staining properties and surgical outcome.

Methods: In a retrospective, non-comparative clinical case series, we analysed 17 consecutive chromovitrectomy interventions for surgery of macular holes, ERMs, vitreoretinal traction syndromes and cystoid macular oedema. Following complete posterior vitreous detachment, BBG was injected into the vitreous cavity at a concentration of 0.25 mg/ml, followed by immediate washout. Main outcome measures were staining properties, visual acuity, central visual field testing and optical coherence tomography (OCT) measurements over a mean follow-up period of 3 months.

Results: ILM staining was somewhat less intensive for BBG than for average indocyanine green (ICG) chromovitrectomy. However, the ILM was removed successfully without additional ICG in 15/17 patients. Postoperative visual acuity was improved in 16/17 patients and remained unchanged in one patient. Central retinal OCT thickness showed a postoperative reduction, with values ranging from +7 to -295 μm (median -89 μm). Neither visual field defects nor any other adverse events were recorded.

Conclusion: BBG permits sufficient staining for safe ILM removal. In this short-term study, good anatomical and functional results were achieved and no adverse events were observed.

Key words: acid blue – BBG – brilliant blue G – chromovitrectomy – Coomassie – ILM peeling – ILM staining – internal limiting membrane biostaining

Introduction

Removal of the internal limiting membrane (ILM) is a technically exigent procedure applied widely in macular surgery in order to relieve tangential traction on the fovea. Main indications include vitreoretinal traction syndromes (VRTS), macular hole (MH) surgery, epiretinal membranes (ERMs) and chronic macular oedema (CMO). Because of its transparency and tenuity, the ILM is barely visible. However, complete ILM removal is critical for surgical success (Machemer 1978). The procedure may be facilitated and its safety improved by providing a clear contrast between the ILM and the remaining retina through the application of vital dyes during surgery, an approach commonly designated as chromovitrectomy (Enaida et al. 2006a, 2006b).

Effective and safe performance of chromovitrectomy for ILM removal warrants the use of a biocompatible, non-toxic dye with maximum – and ideally selective – affinity to the ILM. Theoretical foundations that lay the ground for the search for an adequate substance date to the 1960s and were initially a by-product of research

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involving the idea of enhancing the visibility of retinal breaks through the use of intravitreally injected dyes. Kutschera (1969) performed preliminary studies, systematically screening a range of intravitreally applied dyes for characteristics such as toxicity, reabsorption times and retinal break staining ability.

Following an early report on indocyanine green (ICG)-assisted ILM staining (Kadonosono et al. 2000), ICG-assisted chromovitrectomy gained rapid worldwide acceptance and was found to contribute greatly to ILM-peeling safety and ease. However, initial enthusiasm was somewhat extenuated as numerous reports regarding prolonged intraocular persistence of ICG (Weinberger et al. 2001), potential ICG-mediated dose-dependent toxic effects on various retinal cell populations (Iriyama et al. 2004; Maia et al. 2004; Sato et al. 2006), osmotic effects of the solvent (Stalmans et al. 2002), ICG photosensitization (Kwok et al. 2005; Haritoglou et al. 2006a, 2006b), postoperative visual field defects (Haritoglou et al. 2002; Gass et al. 2003), retinal damage caused by an alteration of the cleavage plane during ILM separation (Gass et al. 2003), optic nerve atrophy

(Ando et al. 2004) and increased incidence of a wide array of anatomical and functional postoperative complications (Nagai et al. 2007) were published.

An alternative dye to ICG may be brilliant blue G (BBG), a synthetic triphenylmethane biostain with an absorption maximum at 584 nm also known as Coomassie or acid blue, which binds non-specifically to all proteins (Enaida et al. 2006a, 2006b). In the European Union, BBG was approved for intravitreal use in June 2007. The present investigation was performed to assess the staining properties of BBG at the level of the inner limiting membrane (ILM) and its safety following use in macular surgery.

Materials and Methods

In a retrospective, non-comparative clinical case series, we analysed the patient records of 17 consecutive chromovitrectomy interventions for MH, ERMs, CMO and VRTS. Patient age ranged from 55 to 85 years; seven patients were female, 10 male.

All 17 eyes of 17 patients underwent routine 20- or 23-gauge vitrectomy, performed by three surgeons

(S.P., U.S., P.B.H.) in two centres (Linz, Austria; Basel, Switzerland) using the OS 3 vitrectomy system (Oertli, Berneck, Switzerland) in combination with a Photon II light source (Synergetics, O'Fallon, MO, USA).

In all patients, BBG was injected into the vitreous cavity after a complete posterior vitreous detachment had been achieved. Immediate wash-out was ensured. Initial additional use of intravitreal Trypan blue was employed in the event of suspected ERMs overlying the ILM, and additional intravitreal ICG was applied if contrast enhancement with BBG alone was deemed insufficient.

BBG was used from ready-to-use 0.5 ml vials at a concentration of 0.25 mg/ml, an osmolarity of 306 mOsm/kg H₂O and a pH of 7.52 (Brilliant Peel[®]; Geuder, Heidelberg, Germany). Surgeons were asked to quantify subjectively the strength of ILM staining, using a scale where 10 would represent the aspect of an average staining during ICG chromovitrectomy and 0 an unstained ILM (Table 1).

All patients underwent baseline and postoperative ophthalmic examination, including intraocular pressure, visual acuity measurements, anterior

Table 1. Overview of the most important clinical facts and subjective surgeon ratings of staining intensity.

Patient	Pathology	Biostains used	BBG staining intensity	Preoperative BCVA	Postoperative BCVA	Postoperative visual field defects	Complications
1	MH	BBG	8	0.20	0.40	No	No
2	MH	BBG	8	0.40	0.60	No	No
3	MH	BBG	9	0.16	0.40	No	No
4	MH	BBG + ICG	3	0.13	0.16	No	No
5	MH	BBG	8	0.10	0.20	Yes	Postoperative retinal redetachment
6	ERM	BBG + TB	8	0.40	0.40	No	No
7	ERM	BBG	7	0.40	0.60	No	Minor intraoperative haemorrhage
8	ERM	BBG	8	0.20	0.80	No	Minor intraoperative haemorrhage
9	ERM	BBG	Not recorded	0.30	0.40	No	No
10	ERM	BBG + TB + ICG	2	0.25	0.30	Yes	No
11	VRTS	BBG	7	0.32	0.33	No	No
12	VRTS	BBG	9	0.20	0.30	No	No
13	VRTS	BBG + TB	8	0.40	0.45	No	Minor intraoperative haemorrhage
14	CMO	BBG + TB	7	0.10	0.20	No	No
15	CMO	BBG	7	0.25	0.30	No	No
16	CMO	BBG	8	0.30	0.40	No	No
17	CMO	BBG	7	0.13	0.20	No	No

BBG, brilliant blue G; BCVA, best-corrected visual acuity; MH, macular hole; ICG, indocyanine green; ERM, epiretinal membrane; TB, trypan blue; CMO, chronic macular oedema; VRTS, vitreoretinal traction syndromes.

segment slit-lamp examination and indirect ophthalmoscopy. A detailed medical and ophthalmological history, including metamorphopsia history, was obtained. Baseline and follow-up optical coherence tomography (OCT) was performed. Pre- and postoperative visual field threshold testing using automated static perimetry (PROGRAM DG2; Octopus, Interzeag, Switzerland or HUMPHREY PROGRAM G32; Carl Zeiss Meditec, Jena, Germany) was performed in all patients. Because of the retrospective design of the study, data beyond the scope of this routine workup are not available, including electrophysiological diagnostics. Average follow-up time was 92 days (range 23–105 days).

To detect an effect over time for BCVA and OCT, a linear mixed-effect model with fixed-effect time and random-effect subject was performed. For BCVA, the differences 2–4 weeks postoperative versus preoperative and 3–4 months postoperative versus preoperative were estimated with a corresponding 95% confidence interval (CI). For OCT, the difference 3–4 months versus preoperatively was estimated with a 95% CI. To compare ILM staining affinities between groups with different underlying pathologies, a non-parametric Kruskal–Wallis test was performed. A p -value < 0.05 was considered as significant (Table 2).

In one patient, tissue harvested during vitrectomy was prepared for ultrastructural analysis (patient 5). The excised specimen was placed immediately into phosphate-buffered 2% glutaraldehyde solution for fixation, followed by post-fixation in osmium tetroxide 2% (Dalton's fixative), dehydration in graded concentrations of ethanol and embedding in Epon 812. Ultra-thin sections of 80 nm were contrasted with uranyl acetate and lead citrate for electron microscopy. Analysis and imaging were performed using a Morgagni

268 (D) electron microscope (FEI Company, Hillsboro, OR, USA). At all times, the tenets of the Declaration of Helsinki were observed.

Results

Based on intraoperative observation and postoperative OCT, perifoveal removal of the ILM was complete in 16/17 (94%) eyes. Electron microscopy was performed in one patient, confirming the presence of the ILM. Differences between baseline visual field measurements and follow-up examinations were recorded in 2/17 patients (12%). A peripheral temporal visual field defect related to a peripheral postoperative retinal redetachment was observed 4 weeks after surgery in a patient who had initially presented with an MH combined with a macula-off retinal detachment. One patient with a postoperative episode of prolonged ocular hypertension, ranging from 21 to 39 mmHg during the first 42 postoperative days, presented with generalized loss of visual field sensitivity 4 weeks postoperatively with a reduction in mean sensitivity (preoperative 23.1, postoperative 20.3) and an increase in mean deviation (2.4 preoperative, 5.2 postoperative). However, isolated scotomas were not observed and loss variance was actually reduced from 6.1 to 5.2. Interestingly, this patient was one of two to whom additional ICG had been applied during surgery because of insufficient ILM staining with BBG alone.

Minor intraoperative haemorrhage during ILM removal was observed in 3/17 patients. Other pertinent postoperative adverse events were not observed during clinical examinations in the follow-up period, including signs of possible BBG toxicity (such as increasing retinal or corneal oedema) or relevant intraocular inflammation. Ophthalmoscopy and

OCT demonstrated the complete anatomical closure of 4/5 (80%) MHs. In one patient, the margins of the MH were found to be reattached postoperatively; however, a small central chorioretinal atrophy, already observed preoperatively, persisted. Complete removal of epiretinal gliosis was assessed in 4/5 (80%) macular pucker patients. In one patient, minimal remaining epiretinal hyperreflexivity was observed on postoperative OCT. The relief of discernible vitreoretinal traction was complete in all three VRTS.

Subjective surgeon ratings demonstrated less intensive ILM staining for BBG than for average ICG chromovitrectomy, ranging from 2 to 9 with an average at 7.1 and a mean of 8. However, staining was sufficient to permit successful ILM removal in 12/17 patients without the use of additional biostains (Fig. 2). Staining of ERMs was not observed. For this reason, Trypan blue was administered in three patients with more extensive ERMs for better recognition of ERMs prior to ILM removal. In two patients, additional ICG was applied for better ILM contrast enhancement. Average ILM staining was found to be similar in all underlying pathologies, although a slight trend towards weaker staining in patients with ERM (average affinity grade 6.25) was observed, compared to patients with MH (average grade 7.2), VRTS (average grade 8.0) or CMO (average grade 7.25). This trend was not statistically significant ($p = 0.511$).

Postoperative best-corrected visual acuity (BCVA) was improved in 16/17 (94%) patients and remained unchanged in one patient. In 11/17 (65%) patients, BCVA improved one decimal line or more. The median BCVA increased from 0.25 (range 0.1–0.4) preoperatively to 0.4 (range 0.16–0.8) postoperatively. Central retinal OCT thickness showed a postoperative

Table 2. Statistical analysis (linear mixed-effect model with fixed-effect time and random-effect subject).

	Comparison	Estimate	Lower 95% CI	Upper 95% CI	p-value
BCVA	2–4 weeks postoperative versus preoperative	0.06	0.01	0.12	0.026
BCVA	3–4 months versus preoperative	0.14	0.07	0.21	< 0.001
OCT	3–4 months versus preoperative	–114.87	–171.24	–58.51	0.0013

BCVA, best-corrected visual acuity; OCT, optical coherence tomography; CI, confidence interval.

reduction, with values ranging from +7 to -295 μm (median -89 μm , average -101 μm). The improvement of BCVA and the reduction of central retinal thickness as measured by OCT were both statistically significant at 2–4 weeks, as well as at 3–4 months postoperatively (Table 2).

Ultrastructural analysis of the ILM specimen obtained during BBG-assisted ILM peeling showed an isolated ILM without any adherent retinal tissue. No major cellular debris was found on the retinal surface, indicating the absence of relevant retinal trauma and no other morphological alterations that one might relate to the adverse effects of BBG could be

identified on the retinal or on the vitreal side of the ILM (Fig. 1).

Discussion

Peeling of the ILM has become a standard procedure for the treatment of MHs, epiretinal gliosis, VRTS and some forms of CMO. Complete removal is critical for surgical success but may be challenging because of ILM tenacity and transparency. ILM contrast may be enhanced through the use of vital dyes during surgery; this widely applied approach, designated as chromovitrectomy, is felt to increase the safety and ease of the procedure by the majority of surgeons.

Until recently, none of the available substances were approved for intravitreal use and their employment as ILM biostains represented an off-label use. While there is consensus that the most widely applied biostain (ICG) has excellent ILM staining characteristics, there is an ongoing debate over increasing evidence linking intravitreal ICG to retinal and optic nerve toxicity. For ophthalmic purposes, ICG is approved by the Food and Drug Administration (FDA) only as an intravenous substance in the context of ICG angiography. However, until recently, no reasonable alternative chromovitrectomy dye was available and the majority of retinal surgeons relied on intravitreal ICG for ILM removal on an off-label basis in spite of the evidence of potential toxic effects, as described earlier.

Lately, a number of reports on the preclinical and clinical testing of alternative substances, including infracyanine green (IfCG) (Gass et al. 2003; Haritoglou et al. 2004), trypan blue (TB) (Veckeneer et al. 2001; Luke et al. 2005), triamcinolone acetonide (TA) (Burk et al. 2003; Jonas et al. 2005; Ruiz-Moreno et al. 2007), Fluorometholone acetate (FMA) (Hata et al. 2007), patent blue (PB) (Hiebl et al. 2005; Luke et al. 2006; Mennel et al. 2006; Maia et al. 2007), bromophenole blue (BrB) (Ozawa et al. 2005; Haritoglou et al. 2006a, 2006b, 2007), sodium fluorescein (SF) (Tamai et al. 1984; Das & Vedantham 2004) and BBG (Enaida et al. 2006a,b; Ueno et al. 2007) have been published. However, among the tested substances, only IfCG and BBG showed satisfactory ILM staining.

BBG is used widely for the detection of proteins separated by polyacrylamide gel electrophoresis (Diezel et al. 1972). It is also certified as a food colourant in many countries, including the European Union, where it was registered as E133. However, BBG is banned as a food additive by national law in many European and non-European countries (Lau et al. 2006).

BBG's potential as an ophthalmic dye was first recognized in 2006. Hisatomi reported good staining ability of low-concentration BBG for the anterior capsule of post-mortem pig's eyes. In a rat model, he found no apparent toxic effect to corneal tissues, ciliary body and lens 2 months

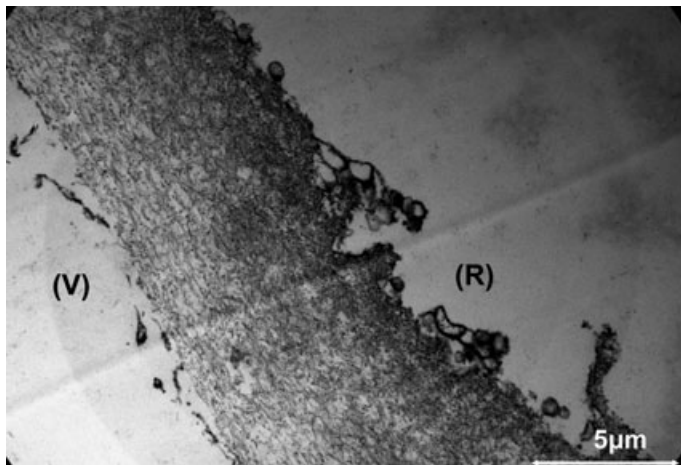


Fig. 1. Specimen shows an electron microscopic section of the ILM after BBG assisted vitrectomy at 28,000-fold magnification. There are no large fragments on the retinal side (R) of the ILM, indicating the absence of major trauma to the underlying retinal structures.



Fig. 2. Intra-operative aspect of an ILM stained with BBG during ILM-removal. A clear contrast to the remaining unstained retina can be appreciated.

after injecting the anterior chamber with BBG solutions of different concentrations and osmolarities (Hisatomi et al. 2006).

BBG's effect on retinal tissues was examined in laboratory animal tests shortly thereafter. Fourteen days after having been injected with BBG solution at several different concentrations, the retinae of Norway rats showed no pathological alterations on light microscopy, only mild vacuolization within the inner retinal cell layers and no signs of apoptosis on electron microscopy (Enaida et al. 2006a). So far, one clinical case series evaluated BBG and found a selective ILM staining mechanism. No adverse events were observed during the perioperative period; visual improvement occurred in 85% of the examined patients (Enaida et al. 2006b). One animal study and one clinical case series had demonstrated good ILM staining abilities, no apparent toxicity and good surgical outcomes prior to CE approval. To our knowledge, further clinical data have not been published yet.

In a retrospective case series of 17 BBG chromovitrectomies, we observed an ILM contrast enhancement somewhat inferior to that of ICG. However, staining was sufficient for safe ILM removal in 15/17 patients. In two patients, additional ICG was used for better ILM staining. ILM staining affinity did not seem to be correlated with the underlying pathology, although there was a trend towards slightly weaker staining in patients with ERF. Postoperative functional outcome was excellent, with BCVA unchanged in patient 6 and improved in all other patients. OCT scans of central retinal thickness were improved in all but one patient (central retinal thickness $+7 \mu\text{m}$ in patient 10). Postoperative visual field defects were observed in two patients. These perimetric defects could be clearly attributed to a postoperative retinal redetachment in one patient. In the second patient, a generalized increase in visual field sensitivity thresholds was observed; this could most likely be explained by a prolonged period of postoperative ocular hypertension. Furthermore, the affected patient represented one of two for whom the use of additional intravitreal ICG had become necessary during surgery for better ILM staining.

In conclusion, BBG has good ILM staining abilities and showed good structural and functional outcomes in our study. We did not find any apparent BBG toxicity. However, one case of generalized postoperative field loss, although possibly explained by postoperative ocular hypertension and the additional use of intraoperative ICG, was observed in connection with intravitreal BBG use. Further investigation with randomized studies is needed to study BBG's potential and safety profile as compared to those of the substances currently in use.

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