Staining of the Internal Limiting Membrane with the Use of Heavy Brilliant Blue G
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Abstract
Background: Brilliant blue G (BBG) is frequently used in chromovitrectomy to facilitate internal limiting membrane (ILM) peeling. A study was initiated to evaluate if heavy BBG is safe and effective in staining the ILM. Methods: We studied 30 eyes, 23 with idiopathic macular holes and 7 of patients with diabetic macular edema. Removal of the ILMs was assisted by heavy BBG staining. In cases with histopathological correlation the ILMs were evaluated with hematoxylin and eosin, Masson’s trichrome, periodic acid-Schiff and glial fibrillary acidic protein staining. In addition, immunohistochemistry was also performed using specific antibodies for vimentin, neuron-specific enolase, factor VIII and CD68. Using the Image-Pro Plus software of Media Cybernetics Co. we found an average thickness in ILMs. Results: Of the ILM specimens sent, 19/30 (63.33%) could not be processed properly because of the limited sample material, recognizing only fragments of dispersed fibrillar material. In macular hole ILMs we found an average thickness of 1.3 ± 0.65 µm, and in diabetic macular edema ILMs an average thickness of 6.2 ± 1.4 µm. Conclusions: In heavy BBG-assisted ILM peeling we observed no intraoperative or postoperative complications after a mean follow-up of 12 months. Heavy BBG could be an effective and safe vehicle for staining the ILM.

Introduction

The internal limiting membrane (ILM) is a transparent, thin structure comprised of the basement membrane of the Müller cells. The Müller cells are essential for the maintenance of the retina’s normal structure and function. Removal of the ILM improves the surgical outcome of several vitreoretinal conditions, including epiretinal membranes, macular edemas, and macular holes [1, 2]. However, difficulties in visualization of the normally completely translucent ILM can increase surgical time and the risk of intraoperative trauma to retinal structures.
Staining of the ILM is widely accepted by ophthalmic surgeons for safer and more convenient removal of the ILM [3, 4]. Indocyanine green and trypan blue staining have greatly facilitated ILM visualization and peeling during vitreoretinal surgery. However, numerous reports have shown the potential toxicity of these dyes in experimental models and in clinical use [5–12], stressing the need for a safe and effective alternative.

The purpose of this study is to report the clinical experience and the histopathological changes of the ILM of eyes submitted to vitrectomy and ILM peeling using heavy brilliant blue G (BBG) as staining agent.

Materials and Methods

Heavy BBG (Brilliant Peel®) was obtained from Fluoron GmbH, Ulm, Germany (via Geuder AG, Heidelberg, Germany). In addition to regular BBG, 13% heavy water (D2O) is included, resulting in a density of 1.018 g/cm³.

All patients had follow-up including best corrected visual acuity, complete slitlamp examination, measurement of central macular thickness using optical coherence tomography (OCT; Topcon 3D OCT 2000), and fluorescein angiography (Topcon TRC 50DX). The OCT was repeated at months 2, 4, 8, and 12, including measuring the macular thickness. The mean follow-up was 12 months.

Posterior vitrectomy was performed on 30 eyes of 30 patients presenting with macular holes and diabetic macular edema. Standard phacoemulsification was performed when needed. Surgery consisted of three-port pars plana vitrectomy with induction of a posterior vitreous detachment by suction using the vitrectomy cutter. BBG, 0.1 ml at a concentration of 0.25 mg/ml, was then gently injected in the vitreous cavity without fluid-air exchange and with the infusion line clamped. Immediately after the injection, the infusion line was opened and the dye was removed via active aspiration with a soft-tip needle (fig. 1a, b). The area of staining was classified according to the size of the stained area: restricted to the 500-µm macular area (grade 1); outside the macula but not exceeding the vascular arcades (grade 2), and staining beyond the vascular arcades (grade 3).

Removal of the ILM was performed using 23-gauge ILM disposable forceps (fig. 1c, d) and the tissue was collected in 10% formaldehyde. ILMs were evaluated using various stains including hematoxylin and eosin, Masson’s trichrome, periodic acid-Schiff and glial fibrillary acidic protein. In addition, immunohistochemistry was also performed using specific antibodies for vimentin, neuron-specific enolase, factor VIII and CD68. In 19 out of 30 cases the ILM could not be processed properly because of the small sample size.

Of the 11 cases that could be processed 5 (45.45%) were from idiopathic macular holes and 6 (54.55%) from diabetic macular edema.

The thickness of the ILM in the evaluated specimens was also measured with the Image-Pro Plus software of Media Cybernetics Co.
**Results**

Between December 2009 and December 2010, 30 eyes of 30 patients were studied. This study complies with the Declaration of Helsinki and the Good Clinical Practice guidelines. All subjects studied gave written informed consent. Of these, 23 had macular holes (12 eyes (52%) stage III, 9 eyes (39%) stage IV, and 2 eyes (9%) with a myopic macular hole). Sixteen patients (70%) were female and 7 patients (30%) were male. The remaining 7 patients (4 males and 3 females) had diffuse diabetic macular edema with a mean central macular thickness of 413 μm on OCT.

The primary closure rate of macular hole after one surgery, as assessed by OCT, was 87% (20 out of 23 eyes). Patients with persistent macular hole underwent a second pars plana vitrectomy and fluid-gas exchange with a 100% macular hole closure rate.

We studied all 30 ILM specimens obtained after pars plana vitrectomy and BBG staining. In 25 eyes the coloring was graded as 3 (83%) and in 5 eyes as grade 2 (17%). The vast majority of membranes studied (28/30, 93%) were visibly ‘wrapped’ by the blue dye. They were included to perform hematoxylin and eosin staining, Masson’s trichrome, periodic acid-Schiff and glial fibrillary acidic protein staining, as well as immunohistochemistry using antibodies specific for vimentin, neuron-specific enolase, factor VIII, and CD68 (fig. 2). Nineteen out of the 30 ILM specimens sent (63.33%) could not be processed properly because of the limited sample material, recognizing only fragments of dispersed fibrillar material. Eighteen (94.73%) were from macular holes and the remaining (5.26%) from diabetic macular edema. A study measuring membrane thickness using the Image-Pro Plus software (Media Cybernetics Co.) found in macular hole ILMs an average thickness of 1.3 ± 0.65 μm, and in diabetic macular edema ILMs an average thickness of 6.2 ± 1.4 μm. ILMs of all eyes showed satisfactory staining despite the immediate irrigation of the dye following instillation.

**Discussion**

BBG, also known as acid blue 90 or Coomassie Brilliant Blue G-250, is a blue dye (color index 42655) with a molecular weight of 854.02 g/mol and the molecular formula of C₃₇ H₄₈ N₃ NaO₇ S₂ (sodium salt). It has long been used for protein staining in the field of biology as it binds nonspecifically to virtually all proteins. In a model of bovine retina, application of BBG at a concentration of 0.25 mg/ml with an exposure time less than 60 s did not cause irreversible effects on the electroretinogram and seems to be safe for intraocular administration [13]. In rat eyes intravitreal doses of BBG up to 10 mg/ml did not cause morphological changes as revealed by light microscopy, but electron microscopy analysis showed cyst formation in the inner retinal cells at concentrations above 1 mg/ml. No apoptosis was detected and no reduction on the electroretinogram waves was noted after 14 or 60 days [14]. In primate eyes the ILM was clearly stained after intravitreal injection of 0.5 mg/ml BBG [15]. Clinical data are consistent with experimental data and suggest that the use of BBG at a concentration of 0.25 mg/ml is safe for clinical use.

Several studies suggested that the ILM plays a significant role in the development and treatment of several macular pathologies such as macular hole and diabetic macular edema [16, 17]. The ILM also plays an important part in macular edema secondary to central retinal and branch vein occlusion [18]. Surgical removal of the ILM has proven to be effective both anatomically and functionally [16]. It is rather challenging to peel and completely remove the unstained ILM completely because of its transparency. Proper visualization via staining not only makes the procedure more successful but also less traumatic.

The use of dyes such as indocyanine green and trypan blue has become widespread to assist in the removal of the ILM and epiretinal membranes. Adverse effects of in-
Indocyanine green on the retina have been reported in several papers [5–10] while trypan blue results in a rather faint staining [11, 12, 19].

Since Enaida et al. [21] described the use of BBG, several reports have reported on its usefulness for staining the ILM [13–15, 20, 22–24]. These studies showed that this dye is not toxic, neither in vivo nor in vitro. No dose-dependent or time-dependent toxicity has been associated with its use. A low concentration is sufficient and obviates the need for fluid-air exchange.

Heavy BBG was created by adding heavy water (deuterium oxide, D₂O), which is water containing the hydrogen isotope deuterium, to a BBG solution in a final concentration of 13%. The osmolarity of the solution is 306 mosm [24]. With this formula of BBG the surgical staining time is shorter compared with standard BBG, reducing both the surgical time and the retinal light exposure.

Gerding et al. [25] reported in an experimental model of hydrodynamics of fluids that heavy BBG had a larger area of staining than traditional BBG.

A second advantage of heavy BBG is its higher-than-water density. The affinity of BBG to proteins may cause staining of other intraocular structures, allowing it to visualize continuous curvilinear capsulorhexis. Inadvertent staining during vitreoretinal surgery of the lens capsule could interfere with visualization issues. Conversely, heavy BBG sinks to the bottom of the vitreous cavity after intravitreal injection and thus avoids direct contact of the dye to the posterior lens capsule, while still allowing for ILM staining.

In our 30 eyes undergoing ILM peeling after heavy BBG staining we observed no intraoperative or postoperative complications after a mean follow-up of 12 months. We therefore conclude that the new heavy BBG could be an effective and safe vehicle for staining the ILM.

**Disclosure Statement**

Proprietary interests: none. Grants: none. Ethical statement: this study complies with the Declaration of Helsinki including current revisions and the Good Clinical Practice guidelines. The procedures followed were in accordance with institutional guidelines and all subjects gave written informed consent before the study. Financial disclosure: none.

**References**


