Double-staining Technique With Brilliant Blue G for Macular Pathology

The dye appears to be safe and effective as an aid for identifying and peeling the ILM.

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Double staining with the vital dye brilliant blue G (BBG) is a useful technique for identifying and peeling the internal limiting membrane (ILM) during vitrectomy in eyes with macular hole or epiretinal membrane (ERM). Some retinal surgeons may be unfamiliar with this technique because they have reservations about use of BBG for staining, or because they do not see the point of double staining. This article reviews some of the evidence suggesting that BBG dye is safe and effective and that the double staining technique can be helpful in ensuring that membranes of interest have been thoroughly removed during vitrectomy.

BBG is widely used outside the United States for staining the ILM in vitreoretinal surgery. A commercial formulation (ILM-Blue, DORC International) is available in Europe but has not received US regulatory approval. The dye has had a complex history in the United States because of problems associated with a compounded formulation.1

Nevertheless, I agree with Steve T. Charles, MD, who has said, “ILM staining is crucial,” and for this indication BBG is “the only safe stain.”2

ASSESSING BBG SAFETY

Several authors have reported that staining with BBG is efficient and safe in animals and in humans at a concentration of 0.025%, although some concerns about retinal toxicity have been raised.

Remy and colleagues3 evaluated the retinal toxicity of BBG following intravitreal injection in rat eyes and found no significant reduction in retinal ganglion cell counts or morphologic alterations. They also assessed the dye’s biocompatibility and staining properties in 18 patients undergoing vitrectomy for macular hole (MH) or epiretinal membrane (ERM) and found that BBG provided selective and sufficient staining of the ILM with no observed retinal toxicity or adverse events.

Enaida and coworkers4 also evaluated the dye in rat eyes. BBG was injected after vitrectomy in rat eyes, and light microscopy and transmission electron microscopy (TEM) were used for analysis. No pathologic changes were seen on light microscopy, but on TEM, with high doses of BBG, vacuoles were observed in the ganglion cell layer. No apoptosis was detected. The authors also evaluated the use of BBG in primate eyes and found that the ILM was clearly visualized and easily peeled off the retina after injection of BBG.

Rodrigues and colleagues5 analyzed the biocompatibility of several dyes, including BBG, in animal eyes. They reported that light microscopy and TEM revealed slight morphologic changes in eyes exposed to 0.05% BBG, similar to control eyes exposed to balanced salt solution, at 1 and 7 days. Electroretinography (ERG) showed intermittent prolonged latency and decreased amplitude in eyes injected with 0.5% BBG, but not the lower 0.05% concentration.

WHY DOUBLE STAIN?

Double staining with BBG was described and clinically evaluated by Shimada and colleagues.6 Patients undergoing vitrectomy for removal of ERM were first stained with BBG,
and the ERM was peeled. BBG stain was then reapplied, and the residual ILM was peeled. The study authors found that BBG staining facilitated simultaneous peeling of the ERM and ILM in many cases, and that double staining resulted in the lowest rate and extent of residual ILM compared with other ERM-peeling methods evaluated. With at least 12 months follow-up, there was no recurrence of ERM (0%) with BBG double staining, compared with 16% recurrence in eyes that underwent single ERM peeling with indocyanine green staining. Postoperative visual acuity did not differ between the 2 methods, suggesting that BBG contact with the retina had no negative effects on visual acuity.

In our own experience, double staining is a simple technique that is especially useful in training fellows. Fellows are inexperienced surgeons who may not see anatomic signs during vitrectomy as readily as more experienced surgeons do. The BBG stain can serve as a clear sign for the surgical trainee. In a macular hole case, for example, the fellow may stain the membrane with BBG, peel it away, and at first be tempted to say, “It was blue, I peeled it, I’m done.” But when we stain it a second time with BBG, the fellow can see that the first membrane was probably the posterior hyaloid, and there is another membrane below (Figures 1 and 2). BBG makes it easy to teach the fellow: When you are not sure you are taking the ILM, stain again.

BBG can also be valuable for ERM removal, even though BBG does not stain the ERM. This depends on the phenomenon we call “negative stain”; that is, the dye stains the ILM around the ERM, allowing the surgeon to clearly see the borders of the ERM (Figure 3). Once the ERM is removed, a second stain then facilitates peeling of the rest of the ILM (Figures 4 and 5).

**SAFETY OF DOUBLE STAINING**

Although the studies cited above have suggested the safety of BBG dye in vitrectomy, the question may arise: If we are staining twice and therefore giving twice the dose, is double staining with BBG safe?

Our center performed a small clinical study to examine...
We included 8 eyes of 8 patients: 6 women and 2 men, average age 68 years. Indication for surgery was macular hole in 5 eyes, ERM removal in 3. After informed consent was obtained, patients underwent pars plana vitrectomy and double staining with BBG at a concentration of 0.025%.

Objectives of this study included anatomic and functional assessment of the effects of BBG double staining. Because animal studies have shown toxicity in the ganglion cell layer with BBG staining, 1 objective was to evaluate the ganglion cell layer in these patients. Imaging was performed preoperatively and at 1 month postoperatively using spectral-domain optical coherence tomography (SD-OCT; Cirrus HD-OCT, Carl Zeiss Meditec) with software that allows anatomic analysis of the ganglion cell layer and the inner plexiform layer. For functional analysis of ganglion cells, pattern reversal ERG testing, specific for measuring the function of the ganglion cells, was performed preoperatively and at 1 month postoperative. Multifocal ERG (mERG) was also performed at the same intervals to measure the function of the macula.

RESULTS AND CONCLUSIONS

On SD-OCT there was no change from preoperative to 1 month postoperative in ganglion cell layer and inner plexiform layer mean thickness. There was also no change in the mean amplitude in the N35-P50 wave or the P50-N95 wave on pattern reversal ERG, and no difference in any of the rings on mERG, from preoperative to postoperative measurements.

The study was small in size and lacked controls. However, no differences that would indicate toxicity or negative effects were seen in the anatomic and functional parameters measured. The study is ongoing, and we will be following patients for up to 6 months, a reasonable length of time to demonstrate any dye-related toxicity.

To summarize the benefits of double staining with BBG: In ERM peeling, BBG staining provides better visualization because of the negative staining effect, and there is less recurrence of ERM after ILM peeling. In vitrectomy for macular hole, BBG provides better visualization, which translates to shorter surgical time and less phototoxicity; BBG also facilitates visualization of ILM remnants on the retina.

Double staining with BBG is a valuable technique for ILM peeling in eyes with macular hole or ERM. It is particularly helpful in the training of new surgeons. It is hoped that a growing body of experience with this technique will continue to show that it is effective and safe for use in multiple clinical applications.

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