Morphological and Functional Outcome after Brilliant Blue G-Assisted Macular Hole Surgery

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Key Words
Macular hole surgery · Dyes · Functional outcome · Primary closure rate · Biocompatibility

Abstract
Purpose of the Study: To investigate the outcome of brilliant blue G (BBG)-assisted internal limiting membrane peeling in macular hole surgery after a follow-up of 6 months. Procedures: In this retrospective study 20 eyes have been treated with BBG-assisted macular hole surgery. After a follow-up of 6 months, the functional and anatomical outcomes have been analyzed. Results: The mean preoperative best-corrected visual acuity (BCVA) was 0.7 logMAR units (mean ± SD 0.66 ± 0.27). After 3 months, the mean BCVA increased not significantly to 0.4 (0.54 ± 0.30), but a significant improvement to 0.2 logMAR units (0.28 ± 0.23) could be detected after 6 months compared to baseline (p < 0.01). Primary macular hole closure after a single surgery was found in 17 of 20 eyes. Conclusion and Message: BBG exhibits sufficient staining qualities and safety profile leading to a significant functional improvement after successful macular hole surgery.

Introduction

Dye-assisted peeling is a milestone in facilitating the visualization of the internal limiting membrane (ILM) during macular hole surgery [1, 2]. The first dye in clinical use was indocyanine green (ICG), which shows a good visualization of the ILM [3], but concerns arose due to a possible critical safety of ICG [4]. In a rat model even a low dose (0.025 mg/ml) of ICG showed functional damage of the retina measured with the electroretinogram [5]. Studies in which retinal glial cells were exposed to ICG support the concerns about functional loss. Cell numbers decreased significantly both when a low dose (0.25 mg) and a clinical dose (2.5 mg) were used [6]. Alternatively, trypan blue (TB) was used in macular hole surgery, but TB showed worse staining potency for the ILM, and is now used more frequently in dye-assisted peeling of an epiretinal membrane [7].

In a comparison of the in vitro toxicity of ICG and TB, TB showed no toxic effects on human retinal pigment
epithelium cells [4, 8]. In another study, however, functional changes were seen after the application of TB. The electroretinogram recorded an irreversible loss of b-waves after exposing retinal cells for a longer time than 15 s [9]. Due to these findings, for macular hole surgery a dye was needed with better staining qualities of the ILM than TB and with a better safety profile compared to ICG. Brilliant blue G (BBG) was introduced in clinical use in 2008 and preclinical data showed a much more comfortable biocompatibility. The intravitreal injection of BBG in rat eyes related neither significant reduction in retinal ganglion cell numbers nor any morphological changes [10]. Only high doses (10 mg/ml and 1 mg/ml) revealed a vacuolization of the inner retinal cells but no apoptotic cell death [11]. Furthermore in comparison with ICG and TB, BBG showed the best biocompatibility data in an ex vivo model of isolated and perfused vertebrate retina [9, 12]. In addition, the application in patients with macular holes and epiretinal membranes showed sufficient and selective staining of the ILM [10, 13]. BBG is now used routinely in macular hole surgery, but only a few studies are available, which reported about the postoperative outcome after BBG-assisted macular hole surgery [14].

Therefore, the aim of this study was to investigate the anatomical and functional outcome after staining the ILM with BBG including a follow-up period of 6 months.

Material and Methods

Patients

Between July 23, 2008, and May 12, 2009, 20 patients with idiopathic macular holes were referred to the University Hospital Schleswig-Holstein, Campus Lübeck, for macular hole surgery. Data collection was performed retrospectively in patients. Inclusion criteria were stages II–IV idiopathic macular holes according to the classification proposed by Gass [15] with a visual acuity of 20/200 or better. Additionally, a follow-up of 6 months should be available. Exclusion criteria were secondary macular holes, previous intraocular surgery, except for uneventful cataract extraction, and ocular disorders that might interfere with vision, such as severe cataract, exudative age-related macular degeneration and diabetic retinopathy. Data analysis was approved by the ethics committee of the Medical University of Lübeck.

Examinations

At baseline, 3 and 6 months after surgery all patients underwent a complete ophthalmological examination including best-corrected visual acuity (BCVA) examination using Early Treatment Diabetic Retinopathy Study charts at a distance of 4 m. Otherwise available decimal VA was converted to logMAR VA. Intraocular pressure measurement was performed using a Goldmann applanation tonometer, slitlamp examination, ophthalmoscopy, optical coherence tomography (OCT; Spectralis OCT, Heidelberg Engineering, Heidelberg, Germany, or the Stratus® OCT, Carl Zeiss Meditec, Dublin, Calif., USA) measuring the central foveal thickness and fluorescein angiography.

Interventions

Patients were included who had received BBG during macular hole surgery and showed an available follow-up of at least 6 months after surgery. The surgical technique included 23-gauge 3-port pars plana vitrectomy and removal of the posterior hyaloid in eyes with stage II–IV macular holes using active aspiration. The amount of 0.1 ml unloaded BBG (Brilliant Peel, Fluoron GmbH, Germany) was applied in a commonly used concentration of 0.25 mg/ml and was removed after 15 s from the posterior pole.

The dye BBG was applied over the posterior pole and under constant irrigation with Ringer solution. The peeling of the ILM extended to an area of at least 2 disk diameters surrounding the macular hole. Then, the peripheral retina was checked carefully for iatrogenic peripheral retinal breaks. At the end, a complete fluid-air exchange was performed including an intraocular gas tamponade (15%) with perfluoroethane (C2F6). Patients were told to maintain the facedown position for 1 week after surgery. There were no differences regarding position, technique, endotamponade, or any other surgical details within the group. All procedures were performed by a single surgeon (S.G.).

Statistical Analysis

For the analysis of the primary outcome variables after dye-assisted macular hole surgery, a nonparametric Mann-Whitney test was applied. The level of significance was p < 0.05. Also for the other statistical analysis, the Mann-Whitney or the Wilcoxon test were used. The data are presented in medians, mean values and standard deviations (SD).

Results

In the BBG group, 20 eyes of 18 patients (4 male, 14 female) were included into the study. For 4 patients no data were available at the 3-month follow-up visit, but for all patients all data were available at month 6. Patients’ ages ranged from 44 to 76 years (65.6 ± 7.4) in the BBG group. The demographic data were summarized in table 1.

Visual Acuity

The primary outcome measure was best-corrected visual acuity (BCVA) at 3 months after macular hole surgery (table 2). We found within the BBG group no statistically significant change between the pre- and postoperative BCVA at 3 months. The mean preoperative VA was 0.7 logMAR units (mean ± SD 0.66 ± 0.27). The mean BCVA at 3 months was 0.4 logMAR units (0.54 ± 0.30), but at this interval only 16 patients of the BBG group could be included in the analysis.
A significant increase in the mean BCVA of 0.2 (0.28 ± 0.23) was present in the BBG group at 6 months compared to baseline (p < 0.01, Mann-Whitney test) (fig. 1, table 2). Eighteen eyes (90%) treated with BBG had a better VA after macular hole surgery and only 2 eyes (10%) decreased.

**OCT and Fluorescein Angiography**

The rate of primary macular hole closure after a single surgery was found in 17 of 20 (85%) examined eyes. Patients with a persisting macular hole underwent a second vitrectomy. One patient in the BBG group showed multiple retinal tears in the periphery with accompanying retinal detachment during the first surgery and showed finally an attached retina under silicone oil tamponade. In the other patients with persisting macular holes, a closure of the macular holes after the second surgery was achieved. One patient with a primary closure did not have complete adjacent edges after 3 and 6 months.

In 16 eyes (80%) we observed cysts in the hole margin preoperatively. Three months after surgery, the number of perimacular cystoid changes was considerably reduced as documented with fluorescein angiography and OCT. Two eyes (13.3%) presented with cystoid changes, 13 eyes (86.7%) showed no edema in the BBG group. Six months after surgery cystoid lesions were identified in 7 eyes (35%). The mean value of the frequency of the development of the macular edema was significantly reduced postoperatively at months 3 (0.13 ± 0.35) and months 6 (0.35 ± 0.49) in comparison to baseline (0.80 ± 0.41, Wilcoxon test, p value 0.002 and 0.007, respectively) (fig. 2). In all patients with persistent cystoid changes other reasons for macular edema such as exudative age-related macular degeneration were ruled out with fluorescein angiography.

**Postoperative Complications**

The most frequent complication was a progression of the cataract. Eighteen eyes developed a cataract and 6 of these received a cataract surgery prior to the 6-month follow-up. One patient suffered from a granuloma by a suture and in 1 patient postoperative intraocular pressure increased to 54 mm Hg and required partial removal of intraocular gas. After partial gas removal the intraocular pressure normalized to 10 mm Hg.

**Discussion**

Our study underlines that BBG appears to be a good alternative dye in macular hole surgery due to its good safety and staining abilities. Our presented data could

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**Table 1. Demographic characteristics of the 20 patients enrolled in the study**

<table>
<thead>
<tr>
<th>BBG group</th>
<th>Eyes, n</th>
<th>Sex, n</th>
<th>Stage of macular hole, n</th>
<th>Lens status, n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 20</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>5 (25%)</td>
<td>Pseudophakic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>15 (75%)</td>
<td>Phakic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>11 (55%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>4 (20%)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cellophane maculopathy</td>
<td>8 (40%)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Clinical outcome after BBG-assisted ILM peeling after 6 months (means ± SD)**

<table>
<thead>
<tr>
<th>Timeline</th>
<th>BCVA, logMAR units</th>
<th>p value</th>
<th>Macular edema</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.7 (0.66±0.27)</td>
<td></td>
<td>0.80±0.41</td>
<td></td>
</tr>
<tr>
<td>Month 3</td>
<td>0.4 (0.54±0.30)</td>
<td>&gt;0.05</td>
<td>0.13±0.35</td>
<td>0.002</td>
</tr>
<tr>
<td>Month 6</td>
<td>0.2 (0.28±0.23)</td>
<td>&lt;0.01</td>
<td>0.35±0.49</td>
<td>0.007</td>
</tr>
</tbody>
</table>
strengthen this assumption, and we found a primary closure rate of 85% in patients who underwent BBG-assisted ILM peeling which was comparable to the closure rate found for ICG and TB [16]. However, in the literature a primary closure rate higher than 90% is reported for BBG-assisted macular hole surgery [17]. Kumar et al. [17] stated that the age of patients who gained anatomical success was significantly lower than in those eyes where closure of the holes did not occur. Patients in that study were 60.8 ± 3.71 years old, whereas the patients in our study were 65.6 ± 7.4 years old. This might explain the lower macular hole closure rate in our case series [18]. Additionally, in our study, macular hole closure was defined as complete closure of the inner retinal dehiscence seen in OCT, which seems to be a very tight definition.

In our study we could demonstrate a significant increase in VA postoperatively. Similar results were reported by Enaida et al. [13] who examined 20 eyes of 20 people who underwent an ILM peeling with BBG. The median visual acuity was 20/100 preoperatively and postoperatively improved to 20/32 [13]. Naithani et al. [18] achieved also a statistically significant improvement in VA.

These results could reflect the good biocompatibility of BBG, which probably leads to a more pronounced significant increase in VA after successful macular hole surgery. In addition, we found a low number of postoperative perimacular cystoid changes in the BBG group, especially compared to the outcome of a prospective study using ICG and TB in macular hole surgery [16]. Although in this study no statistically significant difference was detected for the development of the visual function and for the macular hole closure rate between the ICG and the TB groups, a better visual recovery in the TB group and a higher rate of persistent central scotomata in the ICG group have been observed. The persistent central scotomata could be interpreted as effect of the dye ICG itself which has also been reported previously by von Jagow et al. [19].

Additionally, the observed high incidence of cystoid lesions after dye-assisted peeling in both groups in this prospective study could also represent the toxic effects of the dyes themselves. In consideration of the results found in our study we primarily argue for a better biocompatibility of BBG compared to ICG and TB.

Results of a current meta-analysis revealed that the use of ICG resulted in less VA improvement than other stains, such as TB, and the authors recommended that the toxicity of ICG should be considered when choosing the various staining methods [20]. Therefore, the safety profile of BBG could lead to an at least comparable or even better functional outcome compared to an ICG- or TB-assisted peeling in macular hole surgery which has already been presumed based on previous in vitro studies [11, 13, 21]. Subretinal ICG induces histological and functional damage to the retina, suggesting that ICG should be used with caution in macular hole surgery, where subretinal migration can occur. In contrast, BBG and triamcinolone acetonide appear safe after subretinal injection [22].

Additionally, a current study could demonstrate no significant decrease in the thickness values of the retinal ganglion cell complex and the retinal nerve fiber layer after brilliant blue-assisted ILM peeling in macular hole surgery [23].

Another retrospective study found a better postoperative VA and retinal sensitivity in the central 2° in eyes after BBG-assisted vitrectomy compared to ICG. The restoration of the photoreceptor inner segment/outer segment junction was found to be faster in the BBG group, and the central foveal thickness was significantly thinner in eyes after ICG [24, 25].

In the present study all eyes in the BBG group had undergone no cataract surgery before. After macular hole surgery cataract progression could be observed nearly for all patients. Comparing the cataract development of our study to a prospective study of Beutel et al. [16] we could...
find an equivalent rate of postoperative cataracts, but a slightly higher rate of cataract surgeries was performed during the follow-up in our study (in 6 eyes) compared to the ICG and TB groups of the prospective study. However, in our point of view the higher rate of cataract surgeries in our study should be related to the surgery and not related to the used dye.

Our results were confirmed by a prospective study which compared dye-assisted peeling with ICG, BBG and TB. The researchers found a significantly better functional outcome for BBG compared to ICG, but not compared to TB. However, the concentration of BBG used in this study was 0.05% and is twice the concentration of BBG which is normally used and proposed for macular hole surgery [14].

One reason for its better biocompatibility of BBG may be the advantageous light absorption properties compared to ICG when exposed to the endoillumination during surgery. ICG exhibits infrared absorption properties, with peak absorption at 800 nm. After photoactivation, singlet oxygen and lipid peroxides are developing [26]. An additional direct toxic effect of ICG on the ganglion cells, their axons, and the nerve fibers at the optic nerve head due to its absorption properties has been postulated, but has not been described for BBG so far [27].

However, it has been reported that the staining of the ILM with BBG did not seem to have the same intensity contrast qualities of ICG [28].

A previous study reported on an inadequate removal in 2 of 17 patients without using additional dyes [29]. In our study staining of the ILM succeeded to be atraumatic and easy. Only in 1 patient was the staining of the ILM insufficient. However, newer formulations of BBG with loaded solvent carriers should further improve the staining qualities of BBG [30–32]. In this study only the unloaded formulation of BBG has been used.

In summary BBG seems to be a good alternative dye in macular hole surgery. We have noticed sufficient staining qualities and we have observed a significant functional improvement after macular hole surgery with BBG. Further prospective investigations should be performed to prove our findings which suggest a good retinal biocompatibility and safety profile of BBG which could optimize the procedure of macular hole surgery with regard to safety and functional outcome.

Disclosure Statement

The authors have no financial interest related to the paper.

References


