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ORIGINAL PAPER

Comparison of trypan blue and Brilliant Blue G for staining of the anterior lens capsule during cataract surgery: short-term results

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Abstract

Purpose The present study aimed to evaluate the potential corneal endothelial cell toxicity of trypan blue (TB) and Brilliant Blue G (BBG), two dyes used to stain the anterior capsule in cataract surgery.

Methods We conducted a single-center, prospective, randomized study in which 150 eyes of 117 patients were randomly divided into control (CT), TB, and BBG groups. Preoperative and postoperative (1, 3, and 6 months) values for corrected distance visual acuity (CDVA), corneal endothelial cell count, and central corneal thickness were compared among the three groups.

Results A total of 111 eyes from 88 patients were completely analyzed. The CDVA (logarithm of the minimal angle of resolution) values in the CT, TB, and BBG groups 1 month after surgery were 0.001, 0.023, and 0.019, respectively. The corneal endothelial cell counts 6 months after surgery were 2711 ± 225, 2748 ± 251, and 2680 ± 284 cells/mm^2, respectively. The central corneal thicknesses 6 months after surgery were 524.3 ± 35.5, 532.2 ± 36.1, and 531.4 ± 33.0 μm, respectively. There were no significant differences in CDVA, endothelial cell count, or central corneal thickness among the three groups during the follow-up period.

Conclusions Our findings indicate that neither TB nor BBG was associated with detectable toxicity to corneal endothelial cells during cataract surgery, even when injected directly into the anterior chamber. Additionally, BBG exhibited equivalent staining efficiency to TB.

Keywords Cataract surgery · Trypan blue · Brilliant Blue G · Anterior capsule · Toxicity

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Introduction

Anterior capsule staining during cataract surgery contributes to the successful completion of continuous circular capsulorhexis (CCC) and simultaneously improves surgical outcomes. Historically, indocyanin green (ICG) was first introduced as a dye for anterior capsule staining [1], followed by trypan blue (TB), which is less toxic to the corneal endothelium [2]. However, both ICG and TB have been associated with retinal cell toxicity [3]. In contrast, Brilliant Blue G (BBG) is less toxic to corneal endothelial cells [4–8], although it has mainly been used for staining the inner limiting membrane of the retina during vitreous surgery [9].

Although both TB and BBG exhibit lower cytotoxicity than ICG when used for staining the anterior capsule, few reports have evaluated their efficacy and potential cytotoxicity when staining both the anterior capsule and corneal endothelium during cataract surgery. Therefore, the aim of the present study was to evaluate the efficacy and safety of both stains during cataract surgery by comparing CCC completion and endothelial damage.

Methods

Subjects

The present prospective study was reviewed and approved by the ethics committee of Yokohama Minami Kyosai Hospital (YMH2612) and conducted under the guidelines of the Declaration of Helsinki. After explaining the study to each individual, we obtained written informed consent from that person if he/she met the inclusion criteria.

Standard operating procedures generally reserve staining of the anterior lens capsule for cases in which visualization of the capsule is difficult; this is primarily due to concerns that the corneal endothelium may be damaged by the stain. However, in this study, one of the stains was applied even if the capsule was able to be visualized without it. The hospital IRB approved this protocol after being presented with several published reports, demonstrating that the increased risk of endothelial damage was minimal, since staining of the anterior capsule in cataract surgery is less invasive of the corneal endothelium.

Study plan

We provided a thorough, uniform explanation of anterior capsule staining during cataract surgery to all patients visiting our hospital for cataract surgery consultations. We evaluated data from 150 eyes of 117 patients who underwent cataract surgery at Yokohama Minami Kyosai Hospital between April and September of 2015. Patients were divided randomly into the following three groups for analysis: no staining control (CT), TB, and BBG. A total of 19 eyes in 11 cases (four eyes in the CT group, six eyes in the TB group, and nine eyes in the BBG group) were affected by diabetes. Patients with a history of internal eye surgery such as vitrectomy, glaucoma filtering surgery, or corneal transplant, and those with a history of corneal diseases, such as corneal endothelial inflammation or interstitial keratitis were excluded. Of all the patients receiving consultations during that time, none ($n = 0$) met these exclusion criteria. Additional exclusion criteria included patients with a corneal endothelial count less than 2000 cells/mm^2 as determined during preoperative evaluation ($n = 0$), those with mature cataracts ($n = 0$), and those in whom extracapsular or intracapsular cataract extraction was performed during surgery were also excluded. Cases in which the patient discontinued consultation during the 6 months postoperative follow-up period were also excluded.

Examination

All patients underwent preoperative and postoperative (1, 3, and 6 months) examinations including evaluation of corrected distance visual acuity [CDVA; logarithm of the minimal angle of resolution; (logMAR)], corneal endothelial cell counts (ECDs), and central corneal thickness (CCT). All surgeries were performed by a single surgeon (T.H.). Corneal ECD was measured using the FA-3509 device (Konan Medical, Nishinomiya, Japan). The depth of the anterior chamber and CCT were measured using an SS-1000 OCT device (CASIA®, TOMEY, Nagoya, Japan). The CCC completion rates were also compared among the three groups.

Surgical procedures

Following topical anesthesia using oxybuprocaine hydrochloride (Benoxil® Ophthalmic Solution 4%),

1.0 ml of 2% xylocaine with epinephrine was injected under Tenon's capsule. The concentrations of the BBG (ILM Blue®; DORC, Zuidland, Netherlands) and TB (Vision Blue®, DORC, Zuidland, Netherlands) solutions was 0.1%. Both dyes were obtained by diluting the original solution in balanced salt solution. After creating two paracenteses, 1.0 ml of BBG was injected in the anterior chamber in the BBG group, while 1.0 ml of TB was injected in the TB group. A total of 1.0 ml balanced salt solution was injected in the CT group. Thirty seconds after injection, the anterior chamber was rinsed with 1.5 ml balanced salt solution (Opeguard Neo Kit® Ocular Irrigating Solution 0.0184%, Senju Pharmaceutical Co., Ltd, Osaka, Japan). Ophthalmic viscoelastic devices (OVDs) were then injected into the anterior chamber, and CCC was performed using a 26G cystotome (Terumo Needles® 26G, Terumo Co., Tokyo Japan). A 2.8-mm sclerocorneal incision was then made in the upper sclera. Following hydrodissection, phacoemulsification was performed using the Stellaris PC® (Bausch & Lomb Japan, Tokyo, Japan). The settings on the machine were as follows: Phaco Power 30%, Fixed Pulse mode; linear oscillation; and suction pressure equal to 280–350 mmHg. We employed the soft shell technique [10] using two OVDs [Viscoat® (Alcon Japan, Tokyo, Japan) and Healon® (AMO Japan, Tokyo, Japan)] and the phaco-chop method [11–13] during nucleus processing. After the remaining cortex was aspirated, the lens capsule was expanded using an OVD (Healon®), and an intraocular lens (IOL) was inserted into the capsular bag. Subsequently, the OVD was rinsed with balanced salt solution, and spontaneous wound closure was confirmed.

Statistical analyses

Data are presented as the mean ± standard deviation (SD) or median with interquartile range for continuous variables, or as a frequency with percentage for categorical variables. Baseline characteristics were summarized in accordance with treatment sequences and compared among the three groups using analysis of variance (ANOVA) and post hoc tests for categorical variables as appropriate. Coefficients with 95% confidence intervals (95% CIs) are presented. A P value less than 0.05 was considered statistically significant. All statistical analyses were performed using JMP version 10.1 software (SAS Institute, Inc., Cary, NC).

Results

During the follow-up period, 29 patients (39 eyes) were lost to follow-up (voluntary discontinuation) and excluded from the analysis. In the 29 patients, dropout occurred between 0.5 months and 1.5 months after surgery. Eighteen out of the 29 patients (25 eyes) underwent follow-up observation at the one month postoperative examination, but there were no unusual findings that might indicate a problem forcing their discontinuation. That is, CDVA in these patients was improved over baseline at 1 month, and ECD did not differ significantly compared to those who continued follow-up. No patients had undergone extracapsular cataract extraction/intracapsular cataract extraction. Therefore, 111 eyes of 88 patients were included in the final analysis. Table 1 summarizes the baseline characteristics of the test participants. No significant differences in age, sex, or treated eye were observed among the three groups ($P = 0.68$, 0.82, and 0.84, respectively). The CCC completion rate was 100% in all three groups, and the average CCC size was 5.5 mm in radius. Average phaco times during surgery were 39.73 (CT), 40.23 (TB), and 38.94 s (BBG)

Table 1 Patient characteristics

	Control	TB	BBG	P value
Number of eyes	35	36	40	
Sex (male/female)	13/22	16/20	16/24	0.82*
Age	75.9	75.1	74.5	0.68†
Eye (R/L)	19/16	18/18	19/21	0.84*
CDVA (logMAR)	− 0.16	0.20	0.15	0.43†
ECD	2810	2809	2784	0.84†
CCT	530	533	529	0.88†
Nuclear grade	2.3	2.4	2.5	0.21†

BBG Brilliant Blue G, *CCT* central corneal thickness, *CDVA* corrected distance visual acuity, *ECD* endothelial cell density, *logMAR* logarithm of the minimal angle of resolution, *nuclear grade* was evaluated by Emery retreat classification, *TB* trypan blue

*Chi-square test

†*ANOVA* analysis of variance

($P > 0.01$). Cumulative consumption energy values were 0.0746 (CT), 0.0779 (TB), and 0.0723 Wh (BBG) ($P > 0.05$). No major perioperative complications (e.g., posterior capsule rupture) were observed during surgery.

CDVA values 1 month after surgery were 0.001 (CT), 0.023 (TB), and 0.019 (BBG). In all groups, the CDVA 6 months after surgery significantly improved relative to the preoperative value (all $P < 0.001$, paired t test). CDVA was not significantly different among the three groups during the follow-up period ($P = 0.57$, ANOVA) up to 6 months (Table 2).

Corneal ECDs were 2810 ± 272 (CT), 2809 ± 182 (TB), and 2784 ± 199 cells/mm^2 (BBG) before surgery and 2711 ± 225 (CT), 2748 ± 251 (TB), and 2680 ± 284 cells/mm^2 (BBG) at the 6-month follow-up. The ECD had significantly decreased relative to the preoperative value in each group ($P < 0.001$, 0.040, and 0.017, respectively; paired t test). However, there was no significant difference in ECD among the three groups during the follow-up period ($P = 0.51$, ANOVA; Fig. 1). Furthermore, there was no significant difference in the rate of ECD loss after surgery among the groups (Table 3).

Values for CCT were 523.6 ± 34.3 μm (CT), 533.8 ± 31.5 μm (TB), and 533.4 ± 32.5 μm (BBG) before surgery and 529.7 ± 36.9 μm (CT), 538.3 ± 33.0 μm (TB), and 537.3 ± 34.4 μm (BBG) 1 month following surgery. CCT was significantly increased at the 1-month follow-up compared to the preoperative value in each group ($P = 0.036$, 0.035, and 0.019, respectively; paired t test). However, CCT was not significantly different among the three groups ($P = 0.53$, ANOVA). CCT values at the 6-month follow-up were 524.3 ± 35.50 μm (CT), 532.2 ± 36.05 μm (TB), and 531.4 ± 32.99 μm (BBG), with no significant difference observed among the groups ($P = 0.57$, ANOVA) (Fig. 2).

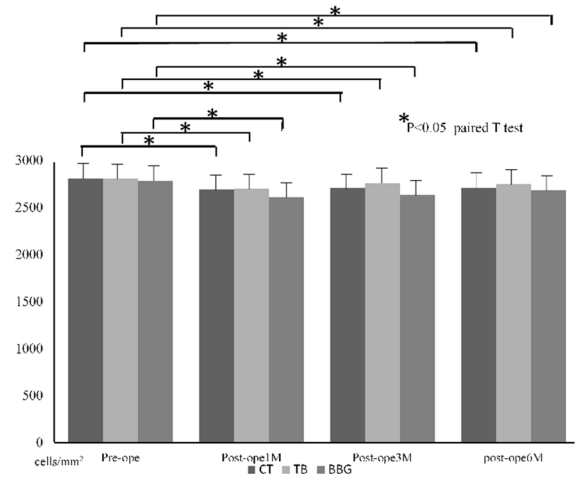

Fig. 1 Comparison of corneal endothelial cell density before and after cataract surgery. No significant differences were observed among the three groups at any time during the follow-up period (*ANOVA* analysis of variance). *CT* control, *TB* trypan blue, *BBG* Brilliant Blue G

Age, gender, history of diabetes, and presence or absence of anterior capsule staining did not affect the change in ECD after surgery (Supplementary Table 1). Similarly, there was no significant influence of age, sex, or history of diabetes on ECD after surgery (Supplementary Table 2) in the anterior capsule staining groups.

Discussion

The purpose of our study was to compare the safety and efficacy of TB and BBG for anterior capsule staining during cataract surgery. We observed no significant difference in ECD or postoperative corrected vision improvement among the groups of the present study, indicating that neither TB nor BBG administered during cataract surgery was significantly

Table 2 Comparison of the pre- and postoperative logMAR ratios among the groups

	Control	TB	BBG	*P* value
Pre-op	0.156 ± 0.154	0.203 ± 0.207	0.152 ± 0.197	0.430
Post-op 1M	0.001 ± 0.095	0.023 ± 0.145	0.019 ± 0.154	0.749
Post-op 3M	− 0.026 ± 0.061	0.029 ± 0.145	− 0.007 ± 0.169	0.217
Post-op 6M	− 0.020 ± 0.070	0.017 ± 0.142	− 0.002 ± 0.196	0.565

The data were compared via an analysis of variance (ANOVA)

BBG Brilliant Blue G, *logMAR* logarithm of the minimal angle of resolution, *TB* trypan blue

Table 3 Postoperative rate of corneal endothelial cell loss (%)

	Control	TB	BBG	*P* value
Post-op 1M	− 4.092 ± 7.256	− 3.774 ± 6.416	− 5.992 ± 14.50	0.594
Post-op 3M	− 6.023 ± 17.47	− 1.713 ± 6.659	− 5.057 ± 12.01	0.326
Post-op 6M	− 3.172 ± 5.860	− 2.195 ± 6.523	− 3.547 ± 9.792	0.737

The data were compared via an analysis of variance (ANOVA)

BBG Brilliant Blue G, *TB* trypan blue

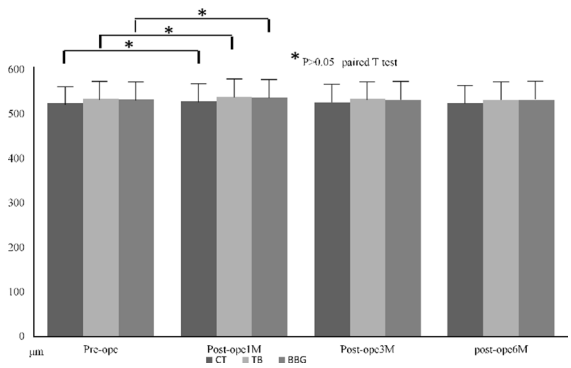

Fig. 2 Comparison of central corneal thickness before and after cataract surgery. No significant differences were observed among the three groups at any time during the follow-up period (*ANOVA* analysis of variance). *CT* control, *TB* trypan blue, *BBG* Brilliant Blue G

toxic to corneal endothelial cells. Our findings suggest that both TB and BBG can be injected directly into the anterior chamber to enhance CCC safety and efficacy. Indeed, we observed that the CCC completion rate was 100% in both the TB and BBG groups. Thus, BBG was both as benign and as effective as TB for anterior capsule staining during cataract surgery.

The phaco-chop method was used for each procedure in the present study, based on the findings of previous reports, which stated that this method is less invasive than other methods in patients with an Emery–Little nucleus grade of 4 or higher [11–13]. There were no cases exceeding nucleus grade 4 in the present study.

Previous researchers have demonstrated the low toxicity of both TB [8] and BBG [14] to corneal endothelial cells during cataract surgery. However, in these studies, the anterior capsule was stained with TB under air or OVD, and the investigators were unable to determine the influence of the dye when the corneal endothelium is directly exposed [14]. Sharma et al. [15] noted that TB is injected directly into the capsular bag for the purpose of performing posterior capsule staining during cataract surgery and reported that the corneal endothelium is undamaged in such procedures. In the present investigation, we injected the diluted stains directly into the anterior chamber. This process allows the dye to directly irrigate the corneal endothelium, thereby potentially influencing cell viability. Regarding BBG, Young et al. [16] suggested that its use in anterior capsule staining may result in the loss of corneal endothelial cells [16]; however, we observed no significant differences in CDVA, ECD, or CCT among the three groups during the follow-up period in this study. Our data are consistent with the results of a study by David et al. [17] and a previous study from this laboratory [18] that reported that corneal endothelial dysfunction is less likely to occur even when TB or BBG is used for graft staining during corneal endothelial transplantation. The results of our current study suggest that neither dye was associated with significant toxicity to endothelial cells and that both can be safely injected directly into the anterior chamber.

In previous studies, the toxicity threshold was noted after 30 min of exposure to 0.1% TB [19]. In another study, the endothelium of human donor corneas was stained for 1 min with 0.3% TB, and no indications of endothelial cell loss in that time span were observed [20]. Dooren et al. [19] also concluded that the toxicity limits in their study allowed for intraoperative TB application. In anterior capsule staining with TB during cataract surgery, a concentration of 0.06% is used to obtain adequate visualization of the anterior capsule in the absence of a red fundus reflex [19]. In our study, we set the concentration of BBG to 0.1%, the value used in vitreous surgery. We observed no difference in toxicity to corneal endothelial cells or CCC completion rate compared with TB. Furthermore, BBG was not inferior to TB regarding anterior capsule visibility after staining. Previous reports have

suggested that BBG is less toxic to the retina than TB or ICG during vitreous surgery [21]. Given these findings, it is likely that BBG can be used to perform cataract surgery more safely than TB, and without any toxicity to the retina even if it flows into the vitreous cavity in the zonule of Zinn.

The present study possessed several limitations. First, we did not measure the change in the ECD and CCT the day after surgery. Second, we did not investigate the impact of the stains in patients with diseases characterized by corneal endothelium vulnerability, such as Fuchs' corneal dystrophy, diabetes. Furthermore, the number of cases examined was relatively small, necessitating additional investigation. Future studies should examine the influence of BBG on the retina via electroretinography (ERG) and optical coherence tomography (OCT).

Nonetheless, the results of our study indicate that BBG exhibits equivalent staining efficiency to TB and that both TB and BBG are non-toxic even when directly injected into the anterior chamber. Although TB and BBG are both safe and effective during cataract surgery, BBG should be used for cases in which the zonule of Zinn has been identified as fragile or compromised, as well as for cases in which simultaneous vitreous surgery is performed. In conclusion, our findings indicate that both TB and BBG are useful for anterior capsular staining during cataract surgery and that these dyes have little influence on corneal endothelial cells, even when directly administered into the anterior chamber.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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