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RETINAL DISORDERS

Comparisons of cone electroretinograms after indocyanine green-, brilliant blue G-, or triamcinolone acetonide-assisted macular hole surgery

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Abstract
Purpose To compare the function of retinal ganglion cells (RGCs) using the photopic negative response (PhNR) in patients who had undergone indocyaine green (ICG)-assisted, brilliant blue G (BBG)-assisted, or triamcinolone acetonide (TA)-assisted internal limiting membrane (ILM) peeling during macular hole (MH) surgery.
Methods Forty-eight eyes of 48 patients with a macular hole were randomly divided into those undergoing ICG-assisted, BBG-assisted, or TA-assisted vitrectomy (n=16 for each group). Full-field cone ERGs were recorded before and 1, 3, 6, 9, and 12 months postoperatively. The amplitudes and implicit times of the a-waves and b-waves and the amplitudes of the oscillatory potentials (OPs) and PhNRs were measured. The mean deviations (MDs) of standard automated perimetry and the best-corrected visual acuity (BCVA) were measured. The circumferential retinal nerve fiber layer (RNFL) thickness was evaluated by SD-OCT.
Results All macular holes were closed with a significant improvement of the BCVA and MD without differences among the groups. There was no significant difference between the preoperative and postoperative RNFL thickness. The implicit times of the a-waves and b-waves were significantly prolonged, and the ΣOPs amplitude was significantly decreased postoperatively in all groups. These ERG changes were not significantly different among the groups. The postoperative PhNR amplitudes were significantly lower in the ICG group than in the BBG or TA group.
Conclusions The results indicate that the PhNR may detect subclinical impairments of RGCs caused by the possible toxic effect of ICG. This finding adds to the data that BBG and TA may be safer than ICG for use during MH surgery.

Keywords Macular hole · Retinal ganglion cell · Photopic negative response · ICG · BBG · Trimacinolone acetonide

Introduction

Kelly and Wendel were the first to use vitrectomy and a gas tamponade in eyes with a macular hole (MH) [1, 2]. To improve the anatomical success and decrease the recurrence rates, peeling of the internal limiting membrane (ILM) has been advocated [3, 4].

Several dyes have been used as vital stains to make the ILM more visible. The first dye used for MH surgery was indocyanine green (ICG) [5, 6], and it was later found to be more effective in staining the ILM than other dyes [7]. However, ICG was shown to be toxic to the retina [8, 9]. At the concentration used during vitrectomy, ICG was shown to damage the retinal ganglion cells (RGCs) in vitro and in vivo [10, 11]. Visual field defects have also been found after MH surgery using ICG [12–14]. Therefore, lower concentrations and immediate washout were used to try to prevent retinal damage by ICG.

Brilliant blue G (BBG) has emerged as an alternative dye that selectively stains the ILM [15, 16]. Preclinical studies using rats and monkeys demonstrated that BBG is less toxic to the retina than ICG [15, 17], although high concentrations or long exposures to BBG can damage the RGCs and retinal pigment epithelial (RPE) cells [11].

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Triamcinolone acetonide (TA) is a steroidal compound that has been used to make the posterior vitreous membrane and ILM more visible [18–20]. Depositing TA particles on the retinal surface can enable surgeons to see where the ILM has been peeled as the area lacking white specks [20]. In addition, TA-assisted ILM peeling has been associated with good postoperative visual acuity [21, 22]. Although TA is toxic for cultured RPE cells [23], in vivo studies have failed to show significant toxicity on retinal neurons [24–26]. TA suspensions, such as Kenacort (Bristol Myer Squibb, New York, NY, USA) with benzyl alcohol as a preservative, are potentially toxic to the retina including RGCs [27, 28]. Thus, a preservative-free TA is commercially available for ophthalmic surgery that is expected to lower the risk of ocular toxicity [29, 30].

The photopic negative response (PhNR) is a negative-going wave that occurs immediately following the b-wave of the cone electoretinogram (ERG), which arises from the neural activity of RGCs [31]. Earlier studies have shown that the PhNR can be used to evaluate RGC function in patients with glaucoma, retinal ischemic diseases, and optic nerve diseases [32–39].

Ueno et al. recorded the cone ERGs of MH patients who had undergone vitrectomy and ICG-assisted ILM peeling, and they demonstrated that the PhNR amplitude was selectively attenuated at 3 months after surgery compared to the preoperative amplitude [40]. Because these patients did not complain of any visual difficulties including visual field defects after surgery, they concluded that the PhNR reduction represented a subclinical impairment of RGCs caused by the toxic effect of ICG and/or mechanical injury of the gas tamponade. They suggested that comparisons between MH surgery with and without ICG would determine which procedure was responsible for the reduction of the PhNR amplitude.

Thus, the purpose of this study was to compare the photopic full-field ERGs including the PhNR recorded from patients before and after ICG-assisted, BBG-assisted, or TA-assisted ILM peeling during MH surgery.

Methods

Patients and surgical procedures

All patients had a comprehensive ophthalmological examination, including measurements of the best-corrected visual acuity (BCVA) with a Snellen chart, slit-lamp biomicroscopy, and indirect ophthalmoscopy. Spectral-domain optical coherence tomography (SD-OCT) was used for staging the MH and confirming the postoperative closure of the MH.

We examined 51 eyes of 51 consecutive patients who underwent vitrectomy with ILM peeling during MH surgery in our hospital from January 2011 to July 2012. All patients did not have any ocular disease other than a MH and cataract. Three patients were excluded because the intraocular pressure was > 30 mmHg postoperatively. The remaining 48 patients consisted of 32 women and 16 men whose mean age was 64.6 ±7.62 (mean±standard deviation) years with a range from 47 to 76 years. Because nuclear cataracts commonly develop after vitrectomy in patients older than 50 years [41], all patients underwent vitrectomy combined with phacoemulcification and aspiration (PEA) with implantation of an intraocular lens (NX-70, Advanced Vision Science, Inc., Coleta, CA, USA).

Preservative-free TA (MaQaid, Wakamoto Pharmaceutical Co., Ltd, Tokyo, Japan) was suspended in 4 ml balanced salt solution (BSS plus, Alcon Japan, Tokyo, Japan) and injected intravitreally in all patients during vitrectomy to make the posterior hyaloid membrane more visible. Prior to the surgery, ICG or BBG were prepared as recommended [6, 16]. Twenty-five milligrams (25 mg) of ICG (Diagnogreen, Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan) was dissolved in 1 ml of sterilized distilled water and then diluted with 9 ml of BSS plus to a final concentration of 2.5 mg/ml (0.25 %). BBG was dissolved in BSS to a concentration of 0.025 %. These dyes were drawn into a 1.0 ml syringe through a sterilized filter (Millex GS filter unit 0.22 μm, Millipore Ireland Ltd., Cork, Ireland). Approximately 0.2 ml of the dye solution was injected intravitreally with a gentle stream directed toward the posterior pole of the eye after removal of the posterior hyaloids membrane. The dye was removed from the vitreous cavity by infusion and aspiration as rapidly as possible. Alternatively, TA was gently injected onto the surface of the retina in the posterior pole, but was not aspirated from the retinal surface.

Then, the ILM was grasped by an ILM forceps and peeled around the macular hole with a size of approximately three disc diameters. Air-fluid exchange was performed followed by an injection of 20 % sulfur hexafluoride (SF_6). All surgical procedures were performed by a single surgeon (SM). Each patient was randomly assigned to either the IGG (n=16), BBG (n=16) or TA (n=16) group. The average operation time was 41.8±6.90 min (mean±SD) for ICG, 40.6±5.86 min for BBG, and 37.1±6.57 min for TA. The differences in the surgical times were not significant.

This research was conducted in accordance with the Institutional Guidelines of Iwate Medical University, and the procedures conformed to the tenets of the Declaration of Helsinki. An informed consent was obtained from all subjects after a full explanation of the nature of the experiments.

Optical coherence tomography (OCT)

Radial 9 mm scans with six lines passing across the fovea were performed at every visit using spectral-domain OCT (SD-OCT, Spectralis, Germany). A closure of the macular

hole was confirmed postoperatively in all patients. The baseline diameters of the MHs were measured in the SD-OCT images of vertical and horizontal scans. The horizontal and vertical values were averaged. The mean diameters of MHs were 620±256 μm (mean±SD), 722±236 μm, and 786±236 μm for the ICG, BBG and TA groups, respectively. The differences in the size of MHs among the groups were not significant ($P<0.100$).

To measure the thickness of the retinal nerve fiber layer (RNFL) around the optic nerve head, we used circular scans of 1.8 mm radius preoperatively and 12 months after the surgery. Each OCT image consisted of 1,536 points along a 360-degree path around the optic disc. The mean RNFL thickness of these points was used for the analyses.

Standard automated perimetry (SAP)

The static visual fields were determined with a Humphrey Visual Field Analyzer (Carl Zeiss Meditec, Dublin, CA, USA) using the Swedish interactive threshold algorithm (SITA) 30-2 preoperatively and at 1, 3, 6, 9, and 12 months postoperatively on the same days as the ERG recordings. The mean deviation (MD) of each studied point was automatically calculated by this program. The MD was defined as the mean of the difference between the measured sensitivity and normal values of age-matched controls embedded in the instrument.

Recording cone electroretinograms

Before the ERG recordings, the pupils were confirmed to be maximally dilated to approximately 8 mm in diameter following a topical application of a mixture of 0.5 % tropicamide and 0.5 % phenylephrine hydrochloride. The full-field cone ERGs were elicited by red stimuli of 1,600 cd/m^2 (λ_{max}=644 nm, half-amplitude bandwidth=35 nm) on a blue background of 40 cd/m^2 (λ_{max}=470 nm, half-amplitude bandwidth=18 nm). The duration of the stimuli was 3 msec. Before beginning the recordings, all subjects were light-adapted by the background light for at least 10 minutes.

The stimulus and background lights were produced by light emitting diodes (LED) embedded in the active contact lens electrodes that illuminated a diffuser for the stimulus and background lights. The intensity and duration were controlled by an electronic stimulator (LS-C, Mayo Co., Nagoya, Japan). The reference and ground electrodes were placed on the middle of the forehead and right ear lobe, respectively. The responses were digitally band pass filtered from 0.5 to 1000 Hz and amplified 10^5 times (Neuropack μ, MEB 9102, Nihonkoden, Tokyo, Japan). Forty to one hundred responses were averaged with an interstimulus interval of 1 second.

The a-wave amplitude was measured from the baseline to the trough of the first negative response, and the b-wave amplitude from the first trough to the peak of the following positive wave. The implicit times of the a-waves and b-waves were measured from the onset of the stimulus to the peak of the waves. The PhNR amplitude was measured from the baseline to the negative trough at a fixed time point after the flash according to the previous reports [34], due to the difficulty in determining the trough when the PhNR amplitude was reduced (Fig. 1). We determined the peak time that produced maximum amplitudes of the PhNR using the method previously reported by Rangaswamy et al. [34]. Briefly, we measured the PhNR amplitude with 5 ms interval in normal subjects and found that it was largest at 65 ms. Therefore, we measured PhNR amplitudes at 65 ms after the stimulus.

The ERGs were digitally filtered between 100 and 1,000 Hz to record the oscillatory potentials (OPs). The amplitudes of OP1, OP2, and OP3 were measured and summed and designated as ΣOPs as reported (Fig. 1) [36].

Statistical analyses

One-way repeated measures ANOVA was used to determine the statistical significance of the functional and anatomical changes with postoperative time. In addition, Bonferroni′s multiple comparison tests were performed after ANOVA as a post-hoc test.

Two-way repeated measures ANOVA was used to compare data between groups, and the Bonferroni post-hoc tests were performed following the ANOVA to determine the statistical significance between paired data at each time point. These analyses were performed using Prism 5.1 (GraphPad Software Inc. San Diego CA). The level of statistical significance was set at $P < 0.05$.

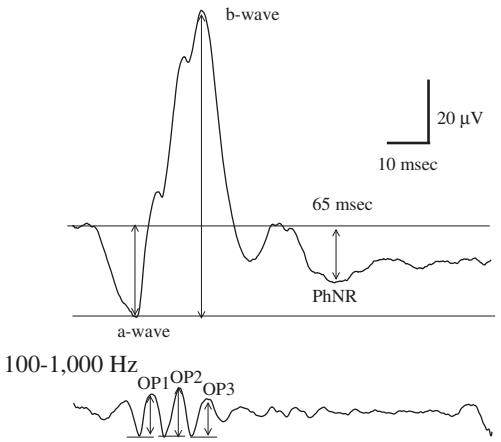

Fig. 1 Photopic ERGs recorded from a patient before surgery. The PhNR amplitude was measured from the baseline to the negative trough at 65 msec after the flash. The amplitudes of oscillatory potentials (OPs) were extracted by digital filtering (100-1,000Hz). The OP1, OP2 and OP3 amplitudes were measured and summed and designated as ΣOPs. ERG: electoretinogram

Results

Changes of BCVA and visual sensitivity

The BCVAs in logarithm of the minimum angle resolution (logMAR) units before and after surgery are shown in Fig. 2a. The mean preoperative BCVA of the ICG group was significantly better that of the TA group ($P<0.05$). The BCVA gradually improved with increasing postoperative times and reached a maximum 9 to 12 months after the surgery ($P<0.0001$). There were no significant differences in the BCVA among the ICG, BBG, and TA groups at each postoperative time. Changes between the baseline and final BCVA were 0.373±0.193 logMAR units (mean±SD), 0.382±0.166 logMAR units, and 0.528±0.241 logMAR units for the ICG, BBG and TA groups, respectively. The differences among the groups were not significant ($P=0.082$).

The differences in the visual sensitivities, represented by the MDs, were not significant at the different preoperative and postoperative times among the ICG, BBG, and TA groups (Fig. 2b). A similar pattern of MD changes was seen for all groups; a gradual increase in the MD with increasing postoperative times ($P<0.001$) and reaching a plateau at around 6 months after the surgery.

Representative ERGs

Representative ERGs recorded from each group before and 1, 3, 6, and 12 months postoperatively are shown in Fig. 3. In all cases, the differences in the amplitudes of the a-waves and b-waves after the surgery were not significant. However, the implicit times of the a-waves and b-waves were significantly longer postoperatively. The OPs were reduced at 1 month postoperatively in all patients; however, they gradually recovered with time.

The PhNR amplitude was significantly reduced at 1 month following surgery in the ICG group ($P<0.01$). With time, the PhNR amplitudes recovered, but they did not return to the baseline level even at 12 months. The PhNR amplitude at 1 month after the surgery in the BBG group was slightly reduced ($P<0.01$), but was followed by a quick recovery to the baseline. In patients in the TA group, there was no change in the PhNR amplitude after the surgery.

Changes of averaged ERGs

Because the ERG amplitudes varied among individuals in the same group, the changes of the ERG amplitudes were expressed relative to the preoperative values to reduce variability among patients in the same group (Figs. 4 and 5). All ERG amplitudes and implicit times are shown as the means and standard errors of the means in these figures. The actual means of the ERG amplitude are given in Table 1 for the a-waves and b-waves, ΣOPs, and PhNR.

There was no significant change in the averaged amplitudes of the a-waves and b-waves after surgery, and there was no difference among the three groups. The implicit times of the a-waves and b-waves were significantly prolonged at 1 month postoperatively in all groups ($P<0.001$, Fig. 4c and d). The implicit times then gradually decreased with increasing time after one month ($P<0.0001$). However, the differences between the groups were not significant at any time points.

To reduce the variations of the ΣOPs and the PhNR amplitudes among individuals in the same group, the ratios of the amplitudes of the ΣOPs to that of b-wave amplitude (ΣOPs/b-wave) and PhNR amplitude (PhNR/b-wave) were also examined (Fig. 5). The ΣOPs amplitude was significantly reduced in all groups at 1 month postoperatively compared to the preoperative values (Table 1, $P<0.0001$). After the reduction

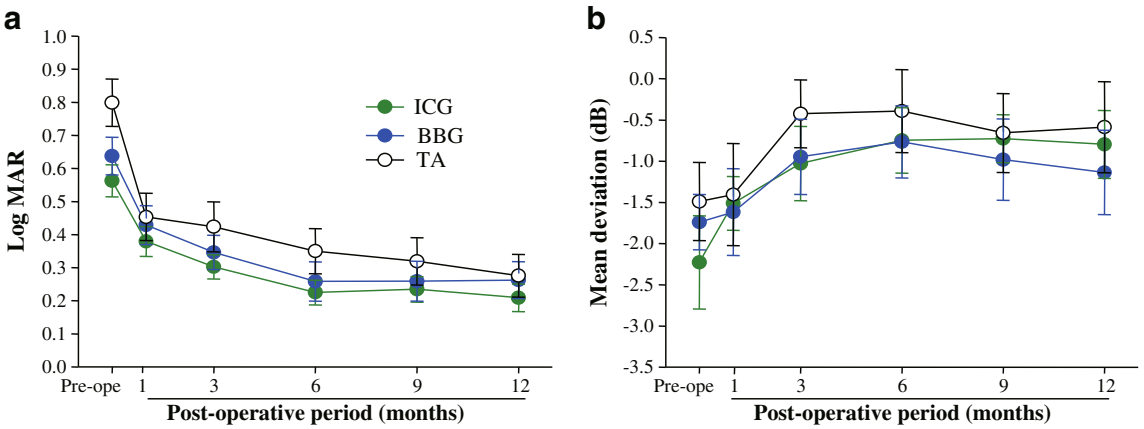

Fig. 2 Best-corrected visual acuity in logarithm of the minimum angle resolution (logMAR) units (**a**) and retinal sensitivity measured by the mean deviation (MD, **b**) of SAP before and after surgery are plotted for ICG, BBG, and TA groups. Green, blue and black symbols represent ICG, BBG and TA groups, respectively. Mean ± SEM. SAP: standard automated perimetry, ICG: indocyanine green, BBG: brilliant blue G, TA: triamcinolone acetonide

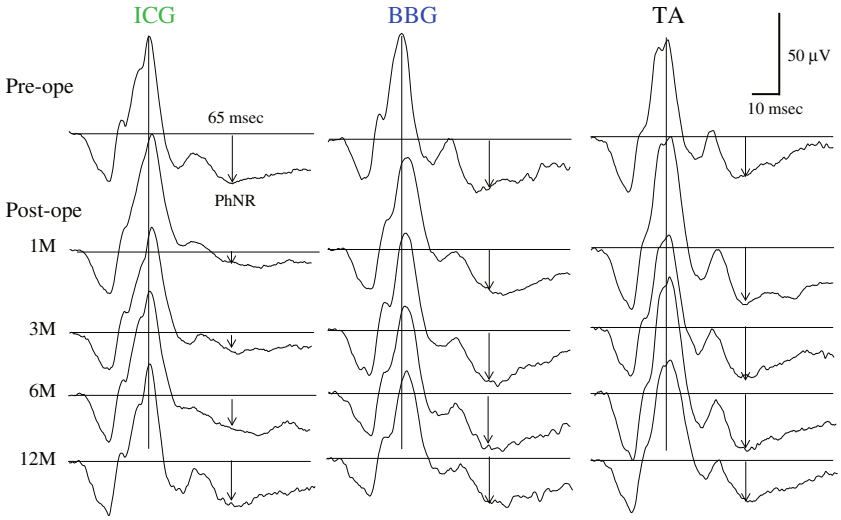

Fig. 3 Representative ERGs recorded from patients of the ICG, BBG and TA groups before and 1, 3, 6 and 12 months after surgery. ICG: indocyanine green, BBG: brilliant blue G, TA: triamcinolone acetonide

at 1 month, the ΣOPs amplitude and ΣOPs/b-wave amplitude ratio gradually but significantly recovered (Fig. 5a and b, $P<0.0001$). However, they did not returned to the baseline level even at 12 months. There was no significant difference in

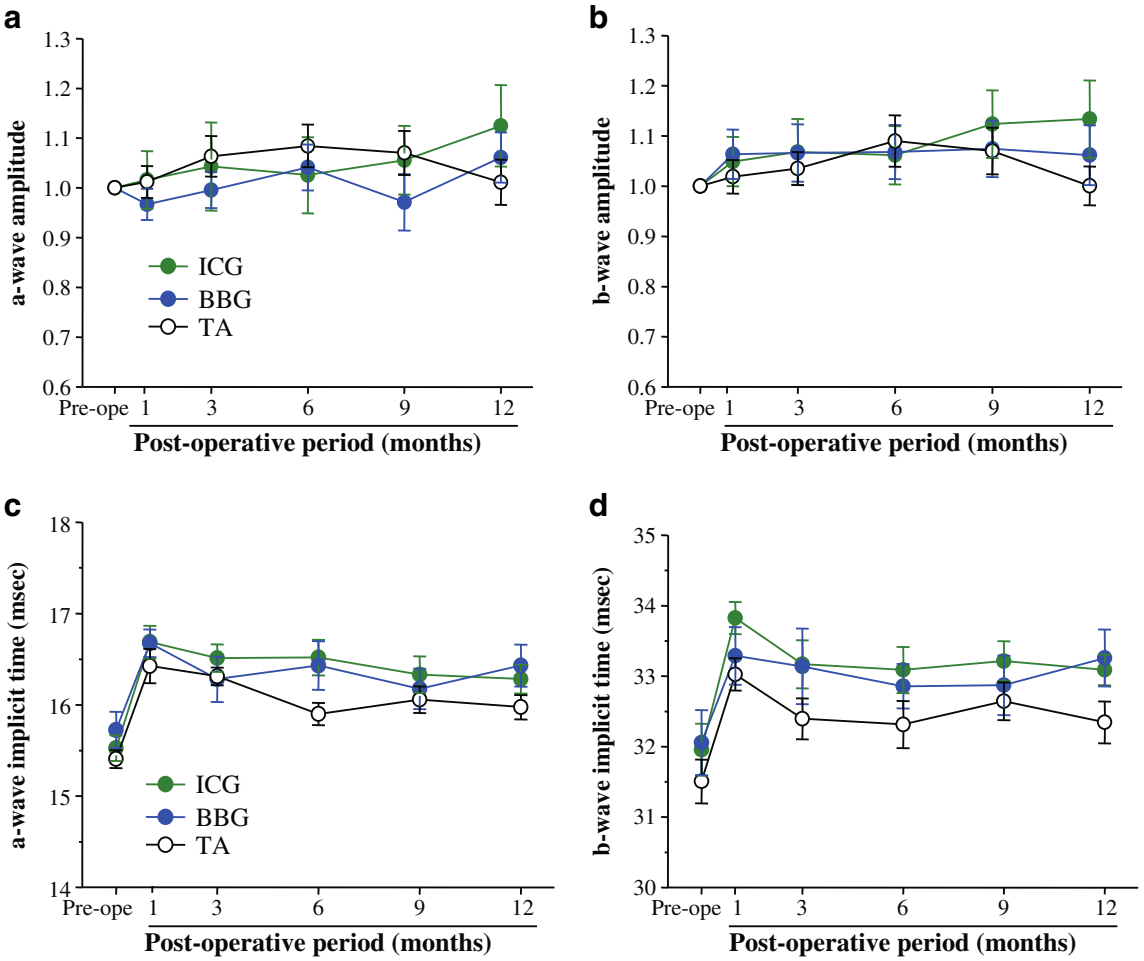

Fig. 4 ERG amplitudes including the a-waves (**a**) and b-wave (**b**) are expressed relative to the to the baseline values and plotted for the ICG, BBG and TA groups before and 1, 3, 6, 9, and 12 months after surgery. Implicit times of the a-waves (**c**) and b-waves (**d**) are also plotted for the ICG, BBG and TA groups. Green, blue and black symbols represent ICG, BBG and TA groups, respectively. Mean±SEM. ICG: indocyanine green, BBG: brilliant blue G, TA: triamcinolone acetonide

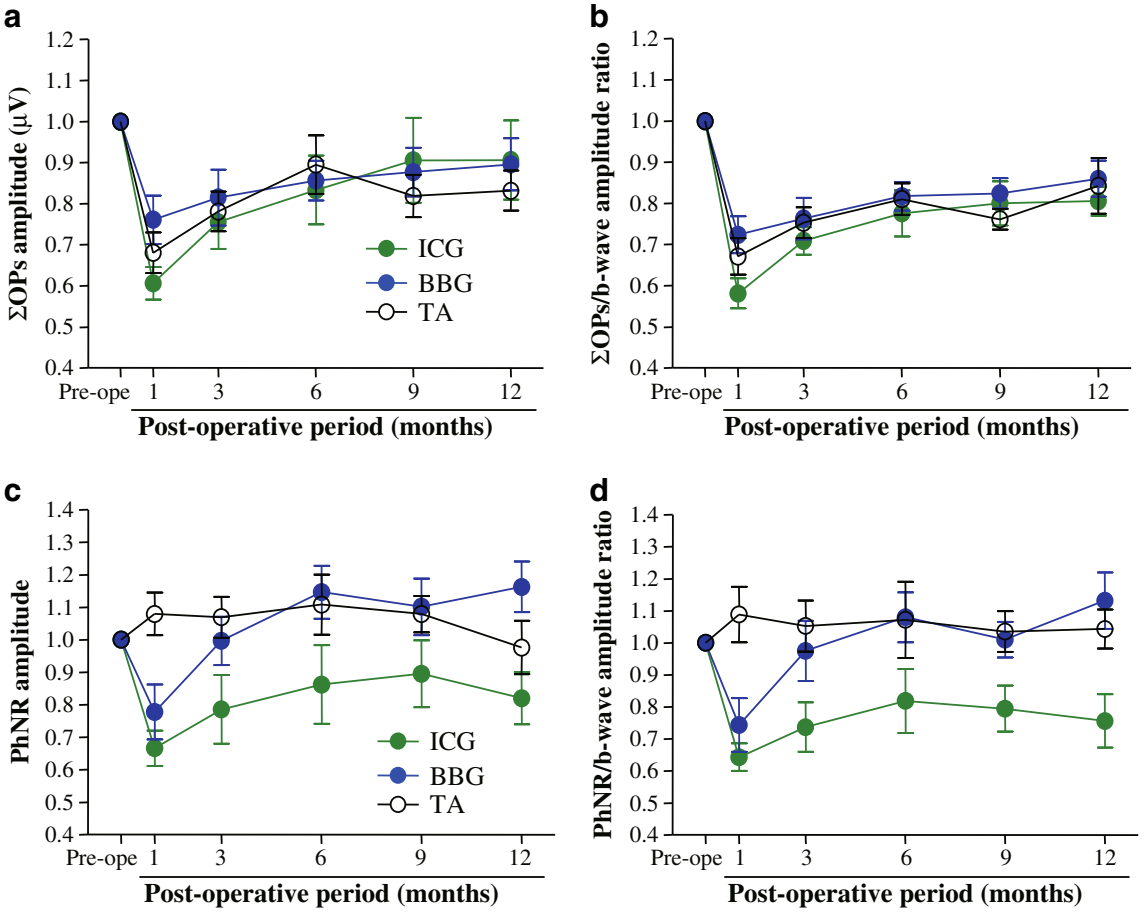

Fig. 5 The ΣOP amplitude (**a**), ΣOP/b-wave amplitude ratio (**b**), PhNR amplitude (**c**) and PhNR/b-wave amplitude ratio (**b**) are plotted against preoperative and postoperative periods for the ICG, BBG and TA groups. Green, blue and black symbols represent ICG, BBG and TA groups, respectively. Mean±SEM. OPs: oscillatory potentials, PhNR: photopic negative response, ICG: indocyanine green, BBG: brilliant blue G, TA: triamcinolone acetonide

the ΣOPs amplitude and ΣOPs/b-wave amplitude ratio among the ICG, BBG and TA groups.

The PhNR amplitude was significantly reduced in the ICG and BBG groups at 1 month postoperatively ($P<0.01$, Table 1). In the ICG group, the PhNR amplitude and PhNR/b-wave amplitude ratio was reduced at 1 month and slowly recovered toward the baseline (Fig. 5c and d). However, they did not reach the baseline level even at 12 months after surgery. A transient decrease of the PhNR amplitude and PhNR/b-wave amplitude ratio was seen in the BBG group at 1 month after surgery. However, there was a rapid recovery to the baseline at 3 months that was different from the ICG group. In the TA group, there was no reduction of the PhNR amplitude and PhNR/b-wave amplitude ratio postoperatively.

Two-way ANOVA demonstrated a significant difference in changes of the PhNR amplitude and PhNR/b-wave amplitude ratio with the postoperative period between ICG and BBG ($P<0.01$ for the PhNR amplitude and $P<0.005$ for the PhNR/b-wave amplitude ratio, post-hoc $P<0.05$ at 12 months), and between the ICG and TA groups ($P<0.0005$, post-hoc $P<0.0001$ and $P<0.05$ at 1 and 3 months, respectively for the PhNR/b-wave amplitude ratio).

Retinal nerve fiber layer (RNFL) thickness

The mean preoperative and postoperative (12 months after surgery) RNFL thicknesses are shown for the ICG, BBG and TA groups in Fig. 6a. There was no significant thinning of the RNFL after surgery in the three groups postoperatively. When the postoperative RNFL thicknesses were compared to the preoperative value, all groups showed minus values with no significant difference among the three groups.

Discussion

We compared the BCVA, retinal sensitivity measured by SAP, and the cone ERGs among patients who had undergone ICG-assisted, BBG-assisted, or TA-assisted MH surgery. Our results showed that the differences in the BCVA, retinal sensitivity, and amplitudes of the ERG components except for the

Table 1 Preoperative and postoperative ERG amplitudes in ICG, BBG and TA groups

	a-wave (μV) (95 % IC)			b-wave (μV) (95 % IC)			ΣOPs (μV) (95 % IC)			PhNR (μV) (95 % IC)		
	ICG	BBG	TA	ICG	BBG	TA	ICG	BBG	TA	ICG	BBG	TA
Preop	36.0 (30.6-41.4)	32.1 (28.5-35.5)	38.7 (36.0-41.4)	110 (93.3-127)	101 (84.3-117)	124 (115-133)	53.1 (39.3-63.2)	43.2 (36.0-50.4)	53.1 (46.7-59.5)	29.5 (26.1-32.9)	26.4 (23.1-29.7)	30.0 (26.1-33.9)
Postop												
1 month	36.2 (30.1-42.3)	30.9 (27.3-34.6)	38.9 (36.1-41.8)	114 (95.2-132)	106 (88.5-123)	126 (114-139)	**30.1** (23.2-37.1)	**32.9** (25.4-40.4)	**35.4** (29.5-41.3)	**19.1** (15.7-22.4)	**21.2** (15.7-26.7)	31.5 (27.6-35.4)
3 months	36.6 (29.9-43.7)	32.0 (27.3-36.7)	40.6 (37.8-43.5)	115 (95.5-134)	106 (87.5-125)	128 (117-138)	**37.1** (29.0-45.3)	**34.2** (27.4-41.0)	**39.9** (35.8-44.0)	23.0 (16.6-29.4)	25.8 (21.4-30.1)	31.5 (26.6-36.5)
6 months	36.1 (29.3-42.9)	32.6 (30.4-34.8)	41.7 (37.7-45.5)	114 (95.9-132)	104 (91.1-117)	134 (120-148)	**40.6** (31.6-49.1)	36.7 (30.0-43.3)	45.7 (38.7-52.7)	24.8 (17.4-32.1)	29.5 (24.6-34.4)	32.3 (27.0-37.5)
9 months	37.9 (29.8-46.0)	32.9 (29.0-37.9)	41.3 (37.2-45.3)	121 (102-141)	107 (88.9-124)	132 (117-147)	42.8 (34.3-51.2)	37.1 (30.2-44.1)	**42.9** (36.1-49.7)	25.4 (19.8-31.1)	28.1 (23.2-32.9)	31.8 (27.7-35.9)
12 months	39.9 (31.3-48.5)	33.9 (29.2-38.7)	38.5 (35.8-41.2)	124 (97.8-148)	105 (88.0-123)	123 (112-135)	45.7 (34.4-57.0)	37.8 (30.7-45.0)	**42.7** (37.6-47.8)	24.2 (18.6-29.8)	29.9 (25.4-34.5)	30.1 (27.0-33.3)

Significant differences in amplitudes compared to the baseline in bold and underlined. *ERG* electoretinogram, *ICG* indocyanine green, *BBG* brilliant blue G, *TA* triamcinolone acetonide, *OPs* oscillatory potentials, *PhNR* photopic negative response

PhNR amplitudes were not significant. However, the postoperative PhNR amplitude was significantly smaller in the ICG group than in the BBG or TA groups. The significantly smaller PhNR in the ICG group indicates a postoperative dysfunction of the retina that was undetectable by other functional measures.

PhNR reduction in ICG group

The PhNR was significantly reduced in the ICG group postoperatively, indicating that the RGC function was depressed. We suggest that this was caused by the toxicity of the ICG. In vitro studies have demonstrated that ICG is toxic to RGCs [11]. Animal studies have shown that intravitreal ICG causes loss of RGCs [10]. These findings support our results in which the PhNR amplitude was reduced in the ICG group.

In addition, the PhNR amplitude did not return to the baseline level even at 12 months after surgery, indicating that ICG most likely damaged the RGCs permanently. This may be because ICG can be taken up by RGCs [42] and remains in the retina for at least 12 months [43–45]. Visual field defects due to RGC dysfunction [46] have been reported to be progressive even 3 years after ICG-assisted MH surgery [14]. These findings suggest that residual ICG could continue to affect RGC function after surgery, possibly explaining the permanent reduction and delayed recovery of the PhNR amplitude in the ICG group.

We used ICG at a concentration of 0.25 % followed by immediate washout; it has been reported that ICG effectively stains the ILM with a concentration as low as 0.05 % [7]. An alternative method of mixing ICG with viscoelastic materials could prevent the ICG from diffusing in the eye and stain the central retina [5]. These procedures could lower the toxic effect of ICG, although further studies are necessary.

Subclinical dysfunction detected by ERGs

It has been reported that the postoperative visual functions including the BCVA and visual sensitivity are worse after ICG than after BBG [22, 47, 48]. However, because the differences were small, other studies have failed to show the toxic effect of ICG on these functional parameters even though a large number of subjects were studied [49, 50].

The functional measures including BCVA and SAP as well as the morphometric parameter such as RNFL thicknesses were not significantly different postoperatively among the groups. It has been reported that the thinning of the ganglion cell complex in the area where the ILM is peeled becomes more apparent after MH surgery without any difference between ICG and BBG or ICG and TA-assisted MH surgery [47, 51]. Therefore, these functional and morphological examinations could not detect the toxic effects of ICG. On the other hands, the PhNR amplitude decreased in the ICG group,

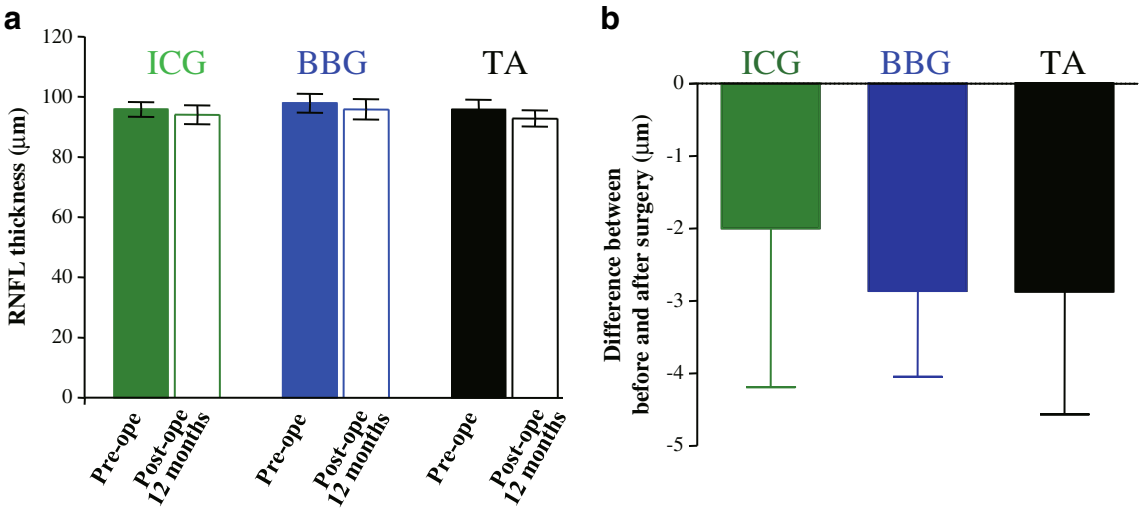

Fig. 6 Histograms demonstrating the preoperative and postoperative retinal nerve fiber layer (RNFL) thicknesses around the optic nerve head for ICG, BBG and TA groups (**a**). Difference between preoperative and postoperative RNFLT(**b**) are shown for each group. Green, blue and black bars represent ICG, BBG and TA groups, respectively. Mean±SEM. ICG: indocyanine green, BBG: brilliant blue G, TA: triamcinolone acetonide

indicating that the PhNR detected a subclinical abnormality of retinal function.

We suggest the following as possibilities for the discrepancy in the PhNR amplitude and other functional parameters. First, SAP measures the retinal sensitivity of the posterior pole of the ocular fundus, whereas the PhNR of the full-field cone ERGs reflects the RGC function of the entire retina including the peripheral retina. ICG may mainly affect the RGC function of the peripheral retina rather than the central retina, because dyes used during surgery were removed by peeling off the stained ILM from the central retina. Second, the visual sensitivity (dB) is usually expressed in logarithmic units, and the relationship between visual sensitivity and PhNR amplitude is curvilinear in glaucoma patients [38, 52]. This indicates that a large reduction in the PhNR amplitude is associated with only a small reduction of the SAP-determined visual sensitivity at the early stage of glaucoma. Because of this relationship, we might overlook the loss of the visual sensitivity. Third, the visual sensitivity is the threshold determined by weak stimuli, while the PhNR amplitude is a response elicited by intense stimuli. In case the intensity-response function is mainly altered in the high intensity range, the PhNR would be decreased without changes in the threshold. This could explain the discrepancy in the results between the PhNR amplitude and visual sensitivity in the ICG group.

There was no significant difference in changes of the RNFL thickness between the before and after surgery values among the groups. The RNFL thickness is related to the number of surviving axons of RGCs. Therefore in the ICG group, it appeared that abnormally functioning RGCs survive without cellular loss, which could explain the PhNR amplitude reduction without thinning of the RNFL thickness in the ICG group.

BBG transiently affects RGC function

The results of at least three animal studies have shown that intravitreal BBG does not affect the retinal structure and function [15–17]. In addition, BBG has neuroprotective properties against photoreceptor cell death [53]. Therefore, most retinal surgeons prefer BBG to ICG for vital staining [47, 48]. However, in vitro safety analysis showed that BBG causes apoptosis and necrosis of RGCs for exposure times longer than 5 minutes [11]. In our results, the PhNR/b-wave amplitude ratio significantly decreased at 1 month after surgery in the BBG group followed by a rapid recovery to the preoperative value. This suggests a transient subclinical alteration of the RGC function induced by intravitreal BBG. Long-term or repeated exposure to BBG may damage the RGC function although BBG is still safer than ICG.

Changes in a-waves and b-waves and OPs

Although it has been reported that the macular function evaluated by the focal macular ERGs improves after vitrectomy for patients with a MH [54], less is known about the postoperative changes of the full-field ERGs which is an indicator of the function of the entire retina. In other words, a decrease in the full-field ERGs would indicate damage to the entire retina rather than the surgical benefits in MH cases, because the treated area is mainly the central retina.

Although the amplitudes of the a-waves and b-waves were unchanged, the implicit times of these ERG components were prolonged followed by a gradual recovery with time. In addition, the ΣOPs amplitudes were significantly reduced but also recovered. These ERG changes were not different among the groups, indicating that the surgical procedures including vitreous and cataract surgeries were the cause of the changes in

the retinal function rather than the vital dyes used during the surgery.

The intraoperative retinal function can be affected by a number of factors, such as temperature of infusion fluid [55], intraocular pressure [56], and light toxicity [57, 58]. In rabbits, it has been shown that a gas tamponade causes degeneration of the outer and inner retina [59, 60]. Glutamate excitotoxicity has been raised as a possible mechanism for the retinal degeneration caused by a gas tamponade [60]. All of these factors could be associated with the ERG changes.

Miyake and Horiguchi demonstrated that the flicker ERGs were reduced and delayed during vitrectomy with subsequent recovery to nearly the preoperative levels at postoperative day 5 [56]. In their patients, long-standing gas that had the potential of damaging the retina [60] was not used in contrast to our study. This could explain why the ERGs had a rapid recovery in their patients.

Why did visual sensitivity measured by SAP improve after surgery?

Because retinal dysfunction would be expected to be localized to the macula in MH patients, it was not expected that the visual sensitivity would gradually improve after the surgery. This is consistent with an earlier report in which the visual fields improved after MH and ERM surgery [61]. The improvements of the visual sensitivity may be due to cataract extraction, because all our cases had cataract surgery. Second, the retinal function gradually recovered from the surgical impairments that were represented by prolongation of the implicit times of the a-waves and b-waves and reduction of the OP amplitudes, which were seen in all groups.

Limitations of this study

A limitation of the current study is that we have not balanced the visual acuity at the entry, which produced the difference in the BCVA at the baseline. This could mask differences in the recovery of BCVA among the groups. We also found differences in the ERG amplitudes among the groups at the baseline. Therefore, we have normalized postoperative ERG amplitudes to the preoperative amplitudes.

Another limitation is that we placed the reference electrode on the forehead, which has a risk of signal contamination by ocular cross-over or from cortical evoked potentials as suggested [62]. However, we used the same procedure to record the ERGs from both eyes simultaneously throughout the study, so that the ERG changes observed in our results could not be attributed to the contamination.

Conclusions

Although there were no significant differences in the BCVA and sensitivities (SAP), the PhNR amplitude was reduced in the ICG and BBG groups postoperatively. A complete recovery of the PhNR amplitude was seen in the BBG group, while the PhNR amplitude did not return to the preoperative level in the ICG group, even at 12 months after surgery. This indicates that the PhNR may detect subclinical impairment of RGCs caused by the possible toxic effects of ICG. This finding adds to the data that BBG and TA may be safer than ICG for use during MH surgery.

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