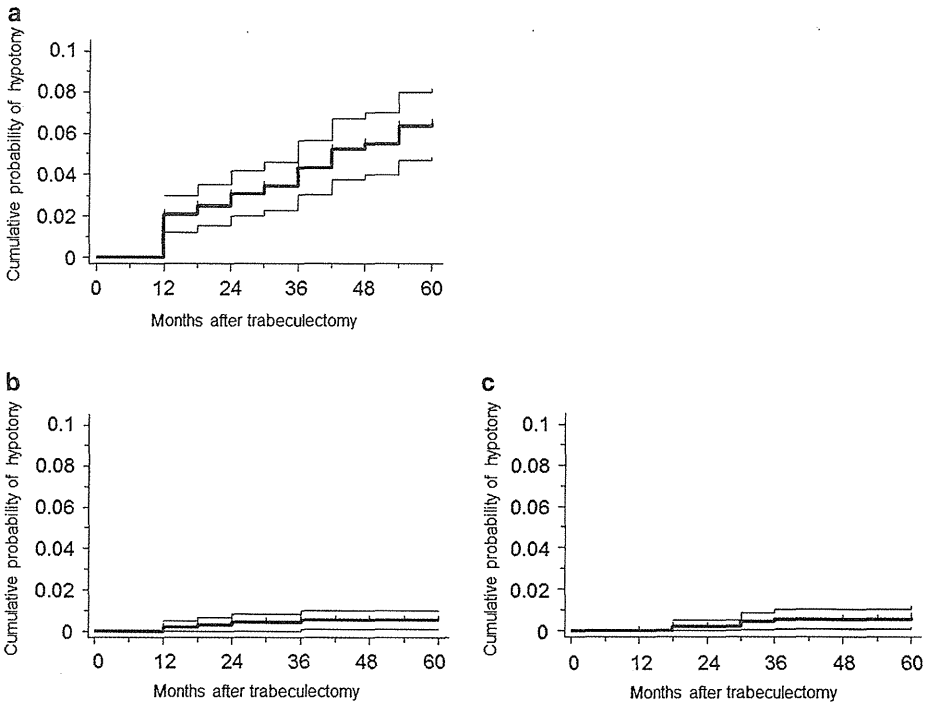

Fig. 4 Kaplan–Meier survival analysis of persistent hypotony. Cumulative probability of persistent hypotony in eyes with sufficient IOP reduction (**a**); eyes that failed on the basis of the criteria of <20 % IOP reduction (**b**) or of further glaucoma surgeries (**c**). The probability is expressed as the proportion to the total number of eyes ($n = 955$). The *thinner lines* indicate the 95 % CIs. No eyes that failed owing to IOP > 21 mmHg developed persistent hypotony

detachment that occurred within 6 months of surgery was larger in the hypotony group than in the nonhypotony group ($P = 0.02$). The follow-up period after trabeculectomy was significantly longer in the hypotonic eyes than in the nonhypotonic eyes ($P = 0.02$).

Results of Kaplan–Meier survival analysis and Cox regression analysis of factors associated with persistent hypotony are shown in Table 3. Multivariate Cox regression analysis showed that preoperative IOP (mmHg), a limbus-based conjunctival flap, and choroidal detachment that occurred within 6 months of the surgery were significant risk factors for persistent hypotony. Every 1-mmHg increase in preoperative IOP reduced the risk of developing persistent hypotony by 5 %.

Bleb infection was noted in 3 eyes with hypotony 46–49 months postoperatively. Of note, persistent hypotony preceded the late-onset bleb infection in all 3 eyes. Two of the 3 infected eyes had anterior chamber involvement, and one had vitreous involvement. The association of bleb infection and visual outcome with persistent hypotony was examined by comparing two groups: eyes with and without hypotony. Because the follow-up period was significantly longer in the eyes with hypotony than in the eyes without (Table 2), and because it was supposedly a confounding variable for both factors, logistic regression analysis was performed with the follow-up period as an additional explanatory variable to adjust for its effect. Bleb infection and final visual acuity (logMAR) were significantly associated with persistent hypotony [logistic regression analysis: odds ratio (OR), 8.74, 1.37; 95 % CI, 1.89–40.4, 1.03–1.82; $P = 0.006$, 0.029, respectively].

Discussion

Persistent hypotony is a representative late complication of trabeculectomy. Theoretically, excess filtration or reduced aqueous humor production, due to inflammation or a compromised ciliary body, underlies the condition. There are two major concerns about the effect of sustained hypotony on the outcome of trabeculectomy: adverse effects on postoperative vision and the influence on surgical success. Although many eyes may tolerate low IOP, persistent hypotony is regarded as an important cause of visual loss after trabeculectomy [3–5]. Hypotony may cause visual impairment through various pathologies, including hypotony maculopathy, accelerated cataract formation, corneal edema, and irregular astigmatism [16, 17]. The incidence of maculopathy in hypotonic eyes varied considerably among previous studies, i.e., 3–38 % [3–5, 18, 19], partly owing to the variability of the definition or the detection modalities used. Optical coherence tomography facilitated the detection of macular chorioretinal folds, even in eyes with subclinical hypotony maculopathy [16]. In our study, hypotony maculopathy was relatively infrequent (5.5 %) in eyes with persistent hypotony.

Table 2 Comparisons of factors between eyes with and without persistent hypotony in cases with sufficient IOP reduction

Factors	No hypotony (630 eyes, 92.0 %)	Hypotony (55 eyes, 8.0 %)	*P* value
Baseline factors			
Age (years), mean, median (range)	63.3, 65.5 (16–88)	61.4, 62 (31–87)	0.11[d]
Sex (male)	379 (60.2 %)	35 (63.6 %)	0.61[e]
Types of glaucoma			
POAG/NTG	301 (47.8 %)	29 (52.7 %)	0.48[e]
Primary angle-closure glaucoma	41 (6.5 %)	1 (1.8 %)	0.13[f]
Exfoliative glaucoma	72 (11.4 %)	7 (12.7 %)	0.77[e]
Neovascular glaucoma	58 (9.2 %)	2 (3.6 %)	0.12[f]
Uveitic glaucoma	73 (11.6 %)	8 (14.5 %)	0.52[e]
Preoperative IOP (mmHg), mean, median (range)	26.7, 24.7 (11.0–70.0)	23.0, 24.0 (11.0–50.0)	0.006[d]
Number of preoperative IOP-lowering medications, mean, median (range)[a]	2.8, 3 (1–5)	2.8, 3 (0–5)	0.73[d]
Preoperative systemic carbonic anhydrase inhibitors[a]	260 (41.6 %)	21 (38.2 %)	0.62[e]
Preoperative BCVA (logMAR), mean, median (range)	0.44, 0.15 (−0.18 to 3.0)	0.52, 0.15 (−0.08 to 3.0)	0.52[d]
Previous cataract surgery	176 (27.9 %)	15 (27.3 %)	0.92[e]
Previous glaucoma surgery	131 (20.8 %)	11 (20.0 %)	0.89[e]
Previous pars plana vitrectomy	32 (5.1 %)	4 (7.3 %)	0.33[e]
Intraoperative factors			
Conjunctival incision (limbus-based)	352 (55.9 %)	42 (76.4 %)	0.003[e]
Concentration of mitomycin C (>0.2 mg/mL)[b]	427 (95.3 %)	24 (88.9 %)	0.15[f]
Duration of mitomycin C application (>2 min)[c]	209 (82.6 %)	13 (76.5 %)	0.36[f]
Simultaneous cataract surgery	104 (16.5 %)	4 (7.3 %)	0.07[e]
Postoperative factors			
Postoperative choroidal detachment (onset, within 6 months of surgery)	37 (5.9 %)	8 (14.5 %)	0.02[f]
Postoperative bleb leak (occurred before persistent hypotony)	50 (7.9 %)	4 (7.3 %)	0.56[f]
Postoperative cataract surgery (performed before persistent hypotony)	49 (7.8 %)	2 (3.6 %)	0.20[e]
Postoperative bleb infection	11 (1.7 %)	3 (5.5 %)	0.10[f]
BCVA at the final follow-up visit (logMAR), mean, median (range)	0.58, 0.22 (−0.18 to 3.6)	0.81, 0.30 (−0.18 to 3.6)	0.15[d]
Change in BCVA (logMAR), final follow-up visit vs baseline, mean, median (range)	0.14, 0.05 (−2.50 to 3.60)	0.28, 0.08 (−0.70 to 2.38)	0.20[d]
Marked decrease in BCVA (>0.3 in logMAR value)	138 (21.9 %)	17 (30.9 %)	0.13[e]
Postoperative follow-up periods (months), mean, median (range)	51.2, 60 (12–60)	56.4, 60 (18–60)	0.02[d]

IOP intraocular pressure, *POAG* primary open-angle glaucoma, *NTG* normal-tension glaucoma, *BCVA* best-corrected visual acuity, *logMAR* logarithm of the minimal angle of resolution

[a] 5 cases were excluded owing to a lack of medication information

[b] 475 cases (69.3 %) were informative

[c] 270 cases (39.4 %) were informative

[d] Mann–Whitney test

[e] Chi-square test

[f] Fisher exact test

In terms of the determination of surgical success, persistent hypotony has been adopted as one of the principal failure criteria of filtration surgery in a number of studies. The guidelines of the World Glaucoma Association recommend setting a lower IOP limit (i.e., 6 mmHg) for IOP success [8]. Although it has been argued that patients with postoperative IOP in the hypotony range are often visually asymptomatic and represent a favorable surgical outcome [20], several studies have pointed out a significant link between hypotony and vision loss [4, 5], providing the

Table 3 Results of Kaplan–Meier survival analysis and Cox regression analysis to identify risk factors for significant hypotony

Factors	No. of eyes[a] (%)	Kaplan–Meier survival analysis	Univariate Cox			Multivariate Cox[b]		
		Log-rank test *P* value	Hazard ratio	95 % CI	*P* value	Hazard ratio	95 % CI	*P* value
Baseline factors								
Age (years)	–	NA	0.99	0.97–1.01	0.41			
Sex (male)	414 (60.4)	0.62	1.15	0.66–1.99	0.62			
Types of glaucoma								
POAG/NTG	330 (48.2)	0.53	1.19	0.70–2.01	0.53			
Primary angle-closure glaucoma	42 (6.1)	0.17	0.28	0.04–2.00	0.20			
Exfoliative glaucoma	79 (11.5)	0.68	1.18	0.54–2.61	0.68			
Neovascular glaucoma	60 (8.8)	0.18	0.40	0.10–1.63	0.20			
Uveitic glaucoma	81 (11.8)	0.58	1.23	0.58–2.61	0.58			
Preoperative IOP (mmHg), median (range)	–	NA	0.95	0.92–0.99	0.007	0.95	0.91–0.98	0.005
Number of preoperative IOP-lowering medications[c]	–	NA	0.99	0.76–1.29	0.96			
Preoperative systemic carbonic anhydrase inhibitors[c]	281 (41.0)	0.63	0.88	0.51–1.51	0.63			
Preoperative BCVA (logMAR)	–	NA	1.24	0.87–1.77	0.24			
Previous cataract surgery	191 (27.9)	0.98	0.99	0.55–1.80	0.98			
Previous glaucoma surgery	142 (20.7)	0.85	0.94	0.48–1.81	0.85			
Previous pars plana vitrectomy	36 (5.3)	0.45	1.47	0.53–4.07	0.46			
Intraoperative factors								
Conjunctival incision (limbus-based)	394 (57.5)	0.004	2.42	1.30–4.51	0.005	2.27	1.21–4.23	0.01
Concentration of mitomycin C (>0.2 mg/mL)[d]	451 (94.9)	0.17[e]	0.44	0.13–1.47	0.19[e]			
Duration of mitomycin C application (>2min)[f]	222 (82.2)	0.55	0.71	0.23–2.18	0.55			
Simultaneous cataract surgery	108 (15.8)	0.09	0.42	0.15–1.17	0.10			
Postoperative factors								
Choroidal detachment[g]	45 (6.6)	0.008	2.63	1.24–5.57	0.01	3.24	1.51–6.95	0.003
Postoperative bleb leak[h]	54 (7.9)	0.90	0.90	0.34–2.60	0.90			
Postoperative cataract surgery[i]	51 (7.4)	0.20	0.42	0.10–1.70	0.22			

CI confidence interval, *POAG* primary open-angle glaucoma, *NTG* normal-tension glaucoma, *IOP* intraocular pressure, *BCVA* best-corrected visual acuity, *logMAR* logarithm of the minimal angle of resolution, *NA* not applicable

[a] Cases with sufficient IOP reduction were analyzed ($n = 685$)

[b] Variables with *P* values <0.05 are shown

[c] 5 cases were excluded owing to a lack of medication information

[d] 475 cases (69.3 %) were informative

[e] Omitted from the multivariate analysis owing to a large number of cases with missing values

[f] 270 cases (39.4 %) were informative

[g] Onset within 6 months of surgery

[h] Occurred before persistent hypotony

[i] Performed before persistent hypotony

rationale for hypotony as a criterion of surgical failure. A significant association was also found between persistent hypotony and worse final visual acuity in our study.

Adding the hypotony criterion to surgical failure decreases the surgical success rate. Bindlish et al. reported that delayed hypotony occurred after a mean follow-up of 26 months in 42 % of 123 eyes with no previous intraocular surgeries and primary trabeculectomy with MMC [3]. The success rate decreased from 83.0 to 57.7 % at year 5 when hypotony and the loss of four or more lines of vison were included in the definition of surgical failure. The authors indicated that eyes with successful IOP reduction at one or more years postoperatively may eventually develop hypotony after a longer follow-up. In the present study, the surgical success at 5 years deteriorated by 6 % when persistent hypotony was considered a failure. The incidence of persistent hypotony may depend on several factors, including the surgical procedure, patient background, length of the study period, and definition of hypotony, which reflects the huge variability in the incidence of persistent hypotony at 5 years: from 1.5 % in the Collaborative Initial Glaucoma Treatment Study to 42 % in the study by Bindlish et al. [3, 4, 18, 21]. The impact of persistent hypotony on surgical success may be better appreciated by drawing separate Kaplan–Meier curves with or without inclusion of hypotony as a failure criterion, especially in a long-term study [6].

Given the related undesirable side effects, the risk factors for persistent hypotony provide vital information for the long-term follow-up of patients after trabeculectomy. Of note, our study was the first long-term, large-scale, prospective study to address the risk factors for persistent hypotony after trabeculectomy with MMC. Multivariate Cox regression analysis identified three independent risk factors: lower preoperative IOP (mmHg), a limbus-based conjunctival flap, and choroidal detachment that occurred within 6 months of the surgery. Regarding the preoperative IOP, every 10-mmHg decrease in preoperative IOP (e.g., 20 vs. 30 mmHg) may increase the risk of developing persistent hypotony by 60 %. The result verified the general concept that eyes with low preoperative IOP, such as normal tension glaucoma, are at greater risk of hypotony given the lower target pressure [2, 22].

As for the type of conjunctival flap, several reports indicated more hypotony in eyes with a limbus-based conjunctival flap [9, 10], and our study results were in line with that conclusion. A limbus-based conjunctival flap showed a greater risk of forming a cystic and avascular bleb [10] accompanied by a larger number of epithelial microcysts on the bleb surface [23], which are considered the channels for the passage of aqueous humor [24]. The morphological differences between blebs with limbus-based and those with fornix-based conjunctival flaps may underlie the likelihood of persistent hypotony. However, another retrospective study reported a controversial result that limbus-based flaps were associated with a lower incidence of symptomatic hypotony [25]. Of note, the subsequent report from the same study group revealed that late low IOP was more common with techniques used by one of two surgeons [19]. Therefore, the surgical procedure may also have a significant impact on the incidence of persistent hypotony.

Choroidal detachment that occurred within 6 months of the surgery was also identified as a risk factor for persistent hypotony. In this regard, Bindlish et al. found that postoperative IOP at month 1 was the single predictor for late-term hypotony [3]. Although postoperative IOP earlier than 6 months was not available in the CBIITS database, the choroidal detachment that emerged within 6 months of the surgery may indicate the presence of overfiltration or low IOP in the early postoperative period.

We also found a significant association between bleb infection and persistent hypotony. Previous studies including the CBIITS indicated that bleb infection may result in bleb scarring and subsequent IOP elevation, possibly owing to inflammation caused by the infection itself or by additional surgeries to treat the infection, or by both [26–28]. Regarding the preinfection status, bleb leakage appeared to be a representative risk factor for bleb infection in multiple studies including the CBIITS [10, 29, 30], so lower IOP was associated with subsequent bleb infection [29, 30]. Poulsen et al. reported that 11 of 20 infected eyes (55 %) had hypotony with Seidel-positive bleb leaks on presentation [31]. However, no previous study has clearly mentioned how many eyes had persistent hypotony before bleb infection. In our study, persistent hypotony was significantly associated with bleb infection. Given that hypotony preceded the late-onset bleb infection in all three eyes that exhibited both hypotony and bleb infection, persistent hypotony may be a predisposing condition for bleb infection. Before infection, an avascular area appeared in all three infected blebs, and bleb leakage was found in one of them.

Regarding the application of MMC, Kupin et al. reported that eyes with MMC exposure (0.5 mg/mL for 3 min) had a significantly higher incidence of prolonged hypotony (IOP < 6 mmHg) than did control eyes (15 vs. 0 % at 9 months, $P = 0.05$) in patients with phakic primary-open angle glaucoma [1]. Regarding normal-tension glaucoma, Membrey et al. reported that eyes with adjunctive MMC more often experienced late hypotony (IOP ≤ 5 mmHg on two successive occasions at least 4 weeks apart) than did eyes with no treatment or adjunctive 5-FU (28 vs. 0 %, $P = 0.002$) [2]. In terms of the duration of MMC application, Zacharia et al. reported an overall 32.7 % incidence of hypotony (IOP < 5 mmHg on 2 visits ≥ 4 weeks apart

and ≥6 weeks postoperatively) and a significantly longer MMC exposure time in eyes with hypotony [32]. However, the association between MMC duration and hypotony was not confirmed in later studies [3, 19]. In our study, the concentration and duration of MMC application were not associated with persistent hypotony.

This study has several limitations. First, since the primary end point was the incidence of bleb-related infection, more than 10 % of cases were excluded owing to incomplete IOP data, and a considerable proportion of the cases were not informative in terms of MMC application, which may have affected the results. Second, the surgical technique and the indication for surgery were not uniform among the centers or surgeons, which may have introduced bias regarding the surgical procedure, e.g., the type of conjunctival flap. This concern may be lessened by the large number of surgeons participating in the CBIITS. Third, there were no standard definitions or diagnosis and treatment procedures for complications. This lack may have resulted in an underestimation of complications such as hypotony maculopathy, and made it difficult to study the reasons for visual loss due to persistent hypotony, although we did find a significant association between persistent hypotony and worse final visual acuity. Despite these limitations, the prospective study design with a large number of cases and a long follow-up period enabled us to successfully identify several factors significantly associated with persistent hypotony after trabeculectomy with MMC.

In conclusion, in the CBIITS, the incidence of persistent hypotony increased over time after successful IOP reduction by trabeculectomy with MMC, and a limbus-based conjunctival flap, lower preoperative IOP, and choroidal detachment that occurred within 6 months of surgery were identified as risk factors for persistent hypotony.

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RESEARCH ARTICLE Open Access

Correlation between optic nerve head circulation and visual function before and after anti-VEGF therapy for central retinal vein occlusion: prospective, interventional case series

Daisuke Nagasato[1,2], Yoshinori Mitamura[3*], Kentaro Semba[3], Kei Akaiwa[3], Toshihiko Nagasawa[1,2], Yuki Yoshizumi[1], Hitoshi Tabuchi[1] and Yoshiaki Kiuchi[2]

Abstract

Background: To determine the correlation between the optic nerve head (ONH) circulation determined by laser speckle flowgraphy and the best-corrected visual acuity or retinal sensitivity before and after intravitreal bevacizumab or ranibizumab for central retinal vein occlusion.

Methods: Thirty-one eyes of 31 patients were treated with intravitreal bevacizumab or ranibizumab for macular edema due to a central retinal vein occlusion. The blood flow in the large vessels on the ONH, the best-corrected visual acuity, and retinal sensitivity were measured at the baseline, and at 1, 3, and 6 months after treatment. The arteriovenous passage time on fluorescein angiography was determined. The venous tortuosity index was calculated on color fundus photograph by dividing the length of the tortuous retinal vein by the chord length of the same segment. The blood flow was represented by the mean blur rate (MBR) determined by laser speckle flowgraphy. To exclude the influence of systemic circulation and blood flow in the ONH tissue, the corrected MBR was calculated as MBR of ONH vessel area – MBR of ONH tissue area in the affected eye divided by the vascular MBR – tissue MBR in the unaffected eye. Pearson's correlation tests were used to determine the significance of correlations between the MBR and the best-corrected visual acuity, retinal sensitivity, arteriovenous passage time, or venous tortuosity index.

Results: At the baseline, the corrected MBR was significantly correlated with the arteriovenous passage time and venous tortuosity index ($r = -0.807$, $P < 0.001$; $r = -0.716$, $P < 0.001$; respectively). The corrected MBR was significantly correlated with the best-corrected visual acuity and retinal sensitivity at the baseline, and at 1, 3, and 6 months (all $P < 0.050$). The corrected MBR at the baseline was significantly correlated with the best-corrected visual acuity at 6 months ($r = -0.651$, $P < 0.001$) and retinal sensitivity at 6 months ($r = 0.485$, $P = 0.005$).

Conclusions: The pre-treatment blood flow velocity of ONH can be used as a predictive factor for the best-corrected visual acuity and retinal sensitivity after anti-VEGF therapy for central retinal vein occlusion.

Trial registration: Trial Registration number: UMIN000009072. Date of registration: 10/15/2012.

Keywords: Anti-vascular endothelial growth factor agent, Central retinal vein occlusion, Fundus-related microperimetry, Laser speckle flowgraphy, Retinal blood flow

* Correspondence: ymitaymitaymita@yahoo.co.jp
[3]Department of Ophthalmology, Institute of Biomedical Sciences, Tokushima University Graduate School, 3-18-15 Kuramoto, Tokushima 770-8503, Japan
Full list of author information is available at the end of the article

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Background

A central retinal vein occlusion (CRVO) is one of the major causes of vision reduction, and anti-vascular endothelial growth factor (VEGF) agents have been shown to significantly improve visual acuity in eyes with macular edema (ME) due to a CRVO [1].

Laser speckle flowgraphy (LSFG) is a non-invasive method of real-time measurements of the blood flow on the optic nerve head (ONH), retina, and choroid [2–6]. It can measure the relative blood flow velocity, called the mean blur rate (MBR), that has been shown to be significantly correlated with the actual blood flow rate determined by the hydrogen gas clearance method and the microspheres technique [7, 8]. It has been reported that LSFG can record the blood flow from the same location of an eye with high reproducibility especially on the ONH [9]. This then provides an accurate way to monitor the circulation changes before and after pharmacological interventions [10].

Yamada et al. reported that the MBR values in large ONH vessels measured by LSFG were correlated with the higher aqueous VEGF concentrations in eyes with CRVO [11]. Nagaoka et al. reported that a single intravitreal bevacizumab (IVB) injection did not affected the retinal microcirculation in eyes with acute branch retinal vein occlusion (BRVO) for at least 3 months after the injection [12]. However, there has been no report that compared the ocular circulation before and after anti-VEGF therapy for CRVO except for a report of 3 cases in which the statistical association between ONH circulation and visual function was not determined [13].

Thus, the purpose of this study was to examine the relationship between the retinal blood flow and visual acuity or retinal sensitivity in 31 eyes before and after IVB or intravitreal ranibizumab (IVR) for CRVO.

Methods

This was a prospective, interventional case series of 31 eyes of 31 treatment-naïve patients (20 men and 11 women) with unilateral CRVO. The patients were examined by LSFG and microperimetry at the baseline and at 1, 3, and 6 months after IVB or IVR for ME due to a CRVO. The fluorescein arteriovenous passage time was determined at the baseline. All patients were examined within 3 month of the onset of the symptoms. The age of the patients at presentation ranged from 40 to 83 years (mean, 66.9 years). All treatment-naïve patients who were diagnosed with ME due to CRVO, had a healthy fellow eye, and had IVB or IVR injections in the Department of Ophthalmology of Saneikai Tsukazaki Hospital from October 2012 through February 2015 were studied. Approval was obtained from the Institutional Review Board of Saneikai Tsukazaki Hospital prior to beginning this study, and the patients gave their written informed consent prior to their inclusion. The patients have provided permission to publish clinical data of their case in this study. This study was registered with the University hospital Medical Information Network (UMIN) clinical trials registry. The registration title is "UMIN000009072, Correlation between optic nerve head circulation and retinal sensitivity before and after anti-VEGF drug intravitreal injection for central retinal vein occlusion" (October 15, 2012). The procedures used in this study adhered to the tenets of the Declaration of Helsinki.

Patients with ME due to a CRVO, central foveal thickness of ≥250 μm in the optical coherence tomographic (OCT) images, and a decimal visual acuity from 0.01 to 0.8 were studied. Patients with a history of cerebral infarction, anti-VEGF therapy, vitrectomy, and uveitis, or other vitreoretinal diseases were excluded. In addition, patients with uncontrolled high blood pressure, diabetes, intraocular pressure (IOP) of 21 mmHg or more, or iris neovascularization were excluded.

The blood flow of the major blood vessels on the ONH was measured in all patients, and the arteriovenous passage time of the retina was determined by fluorescein angiography (FA). A previous study investigating CRVO with FA [14] showed that more than 10 disc areas of the nonperfusion areas were observed in the ischemic type of CRVO. Thus, a diagnosis of ischemic CRVO was made in eyes with more than 10 disc areas of the nonperfusion areas [11]. The venous tortuosity index was calculated on color fundus photograph.

Patients who started their treatment between October 2012 and August 2013 received IVB injections (1.25 mg/0.05 mL), and those who started treatment between September 2013 and February 2015 received IVR injections (0.5 mg/0.05 mL). After the initial treatment, the patients were examined once a month. When the central foveal thickness was ≥250 μm, or the physician determined that additional treatment was necessary, the patients received additional injections of the same anti-VEGF agent.

All patients had a standard ophthalmologic examination including IOP measurements before and after IVB or IVR. The best-corrected visual acuity (BCVA) was measured with a standard Japanese Landolt visual acuity chart, and the decimal visual acuity was converted to the logarithm of the minimal angle of resolution (logMAR) units for statistical analyses. The anterior and posterior segments were examined by slit-lamp biomicroscopy, indirect ophthalmoscopy, color fundus photography, and spectral-domain OCT (SD-OCT). The systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate were measured. The mean arterial pressure (MAP) and the mean ocular perfusion pressure (MOPP) were calculated according to the following formulas and used for the analyses: MAP = DBP + 1/3 (SBP - DBP), and MOPP = 2/3 MAP – IOP.

Laser speckle flowgraphy

LSFG-NAVI (Softcare, Fukuoka, Japan) was used to evaluate the ONH circulation in eyes with a CRVO (Figs. 1 and 2). The principles of LSFG have been reported in detail [15]. In brief, LSFG uses a diode laser (wavelength 830 nm) to detect the movement of the red blood cells in the blood vessels. The light scattered by the movement of the blood cells creates speckle patterns on the area where the sensor is focused, and the scattered light produces a blur in the speckle patterns. The mean blur rate, which is the change in the blur, is a quantitative value that represents the relative blood flow velocity [15–19].

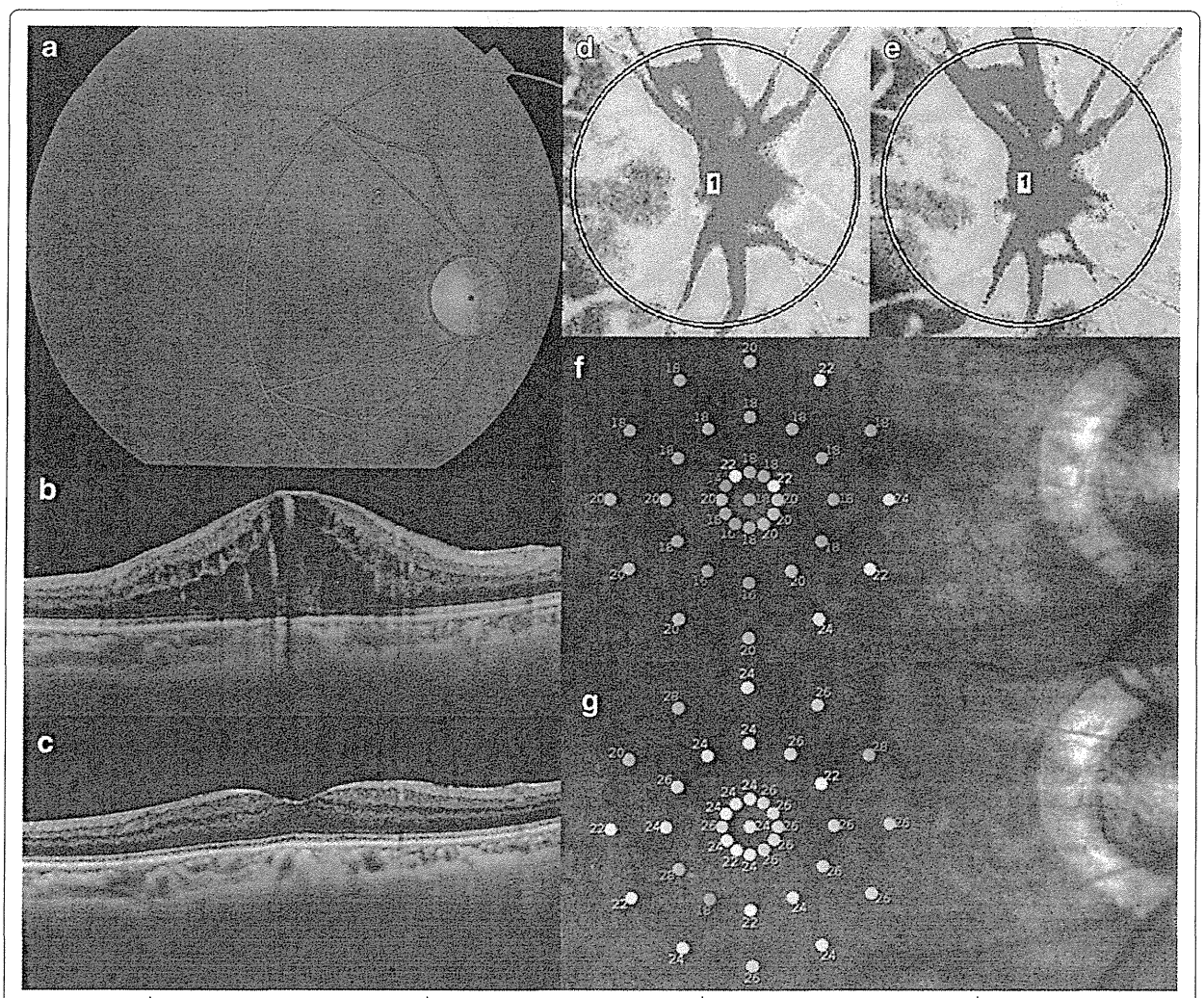

Fig. 1 Ophthalmologic examination images before and 6 months after anti-VEGF therapy for central retinal vein occlusion. Fundus photograph, spectral-domain optical coherence tomographic (SD-OCT) images, laser speckle flowgraphic (LSFG) images, and microperimetric maps before and 6 months after an initial anti-VEGF therapy of the right eye of a 66-year-old woman with cystoid macular edema (CME) due to a central retinal vein occlusion are presented. The decimal best-corrected visual acuity was 0.5 before the treatment and 1.0 at 6 months after the treatment. **a**: Fundus photograph at the baseline. The venous tortuosity index was calculated on color fundus photograph. Measurements of superior and inferior venous arcades were obtained starting from the optic disc margin to the crossing point of a circle whose diameter is the distance from the center of optic disc to the fovea. The course of the veins was traced using Photoshop (Adobe Systems, Inc. Ca, USA). NIH ImageJ software was used to measure the lengths of the tortuous vein (c and d) and chord of the vessels (a and b). The venous tortuosity was calculated by dividing the length of the tortuous retinal vein by the chord length of the same segment (c/a and d/b). The average of the venous tortuosity ((c/a + d/b)/2) was calculated to obtain the venous tortuosity index. **b**: SD-OCT image at the baseline showing CME. **c**: SD-OCT image at 6 months showing a resolution of the CME. **d**: A false-color composite map of the optic nerve head was created using the LSFG findings at the baseline. The red area indicates a faster blood flow, and the blue area indicates a slower blood flow. **e**: A false-color composite map by LSFG at 6 months. There is no obvious difference in the blood flow as compared with the LSFG map at the baseline (**d**). **f**: Microperimetric map image at the baseline. A total of 37 stimulus locations covering the central 10° field were tested. The mean retinal sensitivity at the 37 locations is 19.1 dB. **g**: Microperimetric map image at 6 months. The mean retinal sensitivity at the 37 locations is 24.5 dB

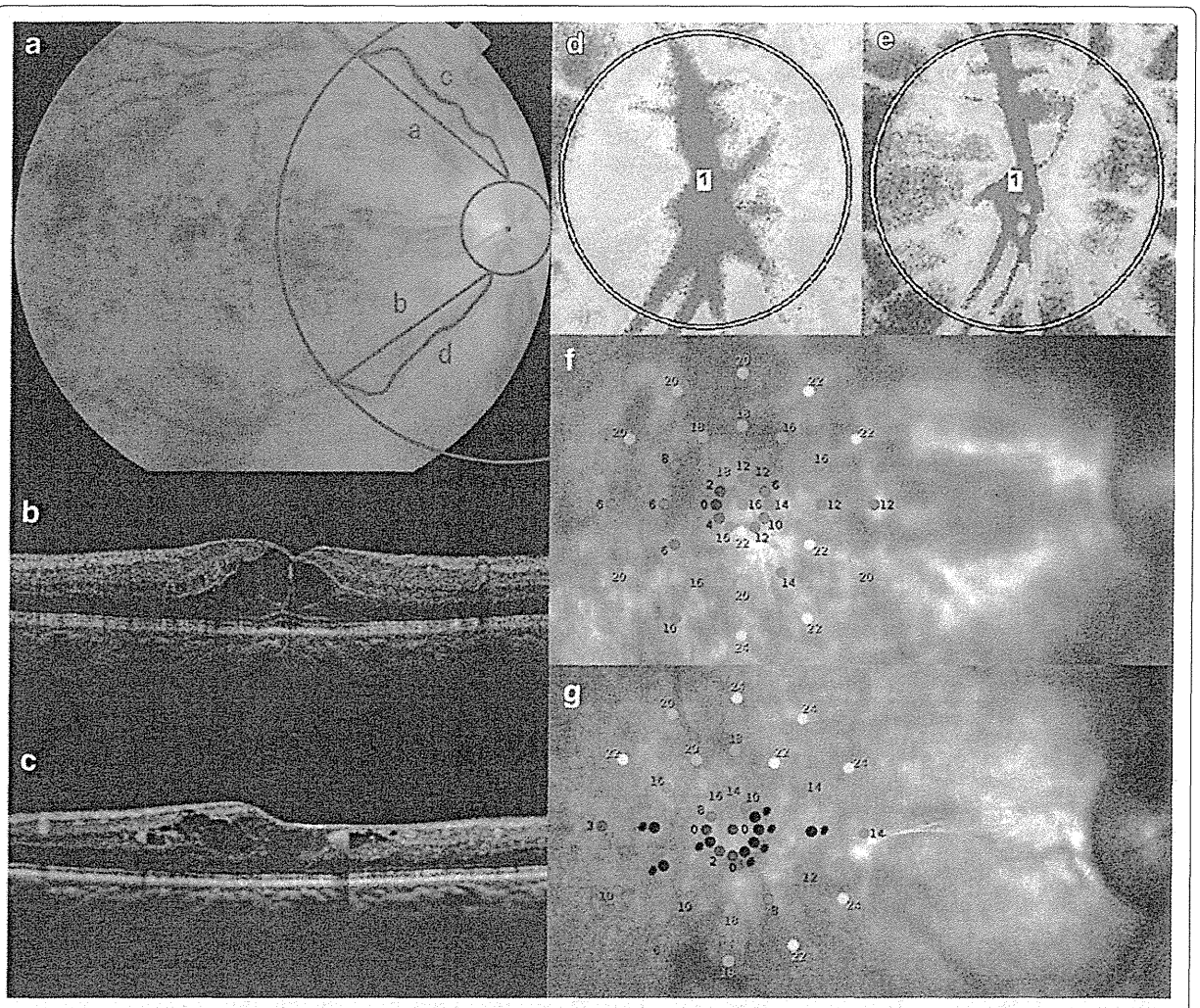

Fig. 2 Ophthalmologic examination images before and 6 months after anti-VEGF therapy for central retinal vein occlusion. Fundus photograph, spectral-domain optical coherence tomographic (SD-OCT) images, laser speckle flowgraphic (LSFG) images, and microperimetric maps before and 6 months after an initial anti-VEGF therapy of the right eye of a 66-year-old man with cystoid macular edema (CME) due to a central retinal vein occlusion are presented. The decimal best-corrected visual acuity was 0.3 before the treatment and 0.1 at 6 months after the treatment. **a**: Fundus photograph at the baseline. Note that the venous tortuosity is larger as compared with fundus photograph presented in Fig. 1a. **b**: SD-OCT image at the baseline shows CME. **c**: SD-OCT image at 6 months indicates residual CME and retinal thinning around the fovea. **d**: A false-color composite map at the optic nerve head was created using LSFG at the baseline. The red area indicates a faster blood flow, and the blue area indicates a slower blood flow. **e**: A false-color composite map by LSFG at 6 months. Note the decrease of blood flow as compared with the LSFG map before the treatment (**d**). **f**: Microperimetric map at the baseline. The mean retinal sensitivity at the 37 locations is 14.4 dB. **g**: Microperimetric map image at 6 months. The mean retinal sensitivity at the 37 locations is 10.6 dB

The patient's pupil was dilated with Mydrin P (1 % tropicamide and 2.5 % phenylephrine) 30 min prior to the LSFG examinations. During the examinations, the patients were instructed to fixate steadily on a target light while the speckle pattern on the ONH was recorded. With an auto-tracking system, the same site can be measured for several seconds. The relative blood flow velocity is represented by the MBR and displayed as a two-dimensional color map. The MBR of the vessel area is expressed as the MV and the MBR of the tissue area as the MT. The appropriate threshold between the vessel and tissue areas is automatically determined by the built-in software, and the MBR of the area of the major arteries and veins as MV and the MBR of the tissue area as MT can be calculated (Fig. 3) [9].

When measuring the retinal circulation with LSFG, the measurements are influenced by the deep choroidal circulation. Similarly, the ONH measurements are influenced by the blood flow of the ONH tissue (MT) [15, 16]. To evaluate the major arteriovenous circulation

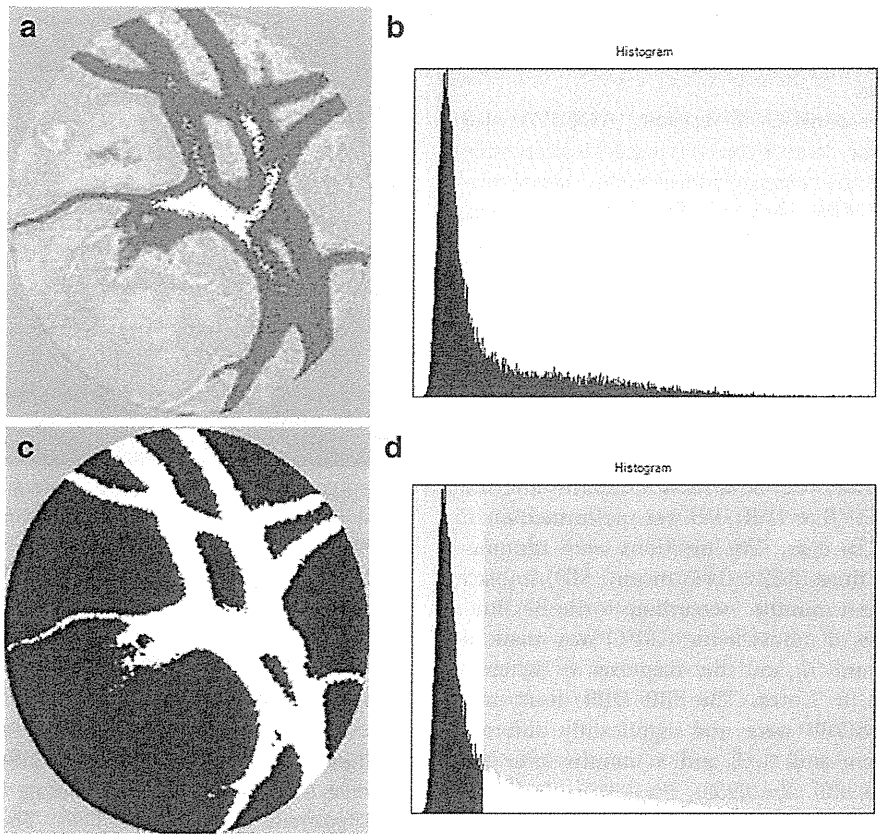

Fig. 3 Composite maps and histograms at the optic nerve head (ONH) created by laser speckle flowgraphy. **a**: A false-color composite map at the ONH. **b**: A histogramic analysis of the ONH. The vertical axis represents the number of pixels and the horizontal axis represents the mean blur rate. **c**: A binary format image for segmentation between the vessel (white area) and tissue (black area) areas. **d**: A histogram analyzed using the built-in image viewer software that uses an automated definitive threshold. The area to the left of the threshold line corresponds to the ONH tissue (black area in **c**), whereas the area to the right of the line corresponds to the ONH vessel (white area in **c**)

of the ONH excluding the blood flow of the ONH tissue, the value was obtained by subtracting the MT from the MV [11]. In addition, to exclude the influence of each patient's systemic circulation at the time of the measurements and to allow for inter-patient comparisons of the measurements, a corrected MBR value was obtained by the MBR (MV minus MT) of the affected eye divided by the MBR (MV minus MT) of the fellow eye [11]. The LSFG was measured at the baseline and at 1, 3, and 6 months after the initial treatment.

Microperimetry

The retinal sensitivity was determined by macular integrity assessment (MAIA) microperimetry (CenterVue, Padova, Italy; Figs. 1 and 2). MAIA testing was conducted in a dark room without pupil dilation. The testing conditions were similar to that described in detail in previous studies [17, 18]. Briefly, 37 loci in the 10° central macula was assessed using a 4 to 2 threshold strategy, a fixation target that consisted of a red circle with a 1° diameter, stimulus size of Goldmann III, background luminance of 4 apostilb (asb), maximum luminance of 1000 asb, and a stimulus dynamic range of 36 dB.

Calculation of venous tortuosity index

The venous tortuosity index was calculated as previously reported (Figs. 1 and 2) [20, 21]. Briefly, color fundus photographs were obtained with a Topcon fundus camera (TRC-50DX, Topcon, Tokyo, Japan). The course of the veins was traced using the Photoshop software (Adobe Systems, Inc. Ca, USA). Measurements of the superior and inferior venous arcades were obtained starting from the ONH margin to the crossing point of a circle whose diameter was the distance from the center of ONH to the fovea. NIH ImageJ software was used to measure the length of the tortuous vein and length of the chord of the vessel. The venous tortuosity was calculated by dividing the arc length of the retinal vein by the chord length of the same segment. The average of the venous tortuosity of the superior and inferior venous

arcades was calculated to obtain the venous tortuosity index.

Statistical analyses

Repeated-measures analysis of variance (ANOVA) with Greenhouse-Geisser corrections was used to determine the significance of the changes in the BCVA, retinal sensitivity, corrected MBR, MV, MT, SBP, DBP, heart rate, IOP, MAP, and MOPP. The Bonferroni test was used for post hoc analysis. Pearson's correlation tests were used to determine the significance of correlations between the corrected MBR and the BCVA, retinal sensitivity, arteriovenous passage time, or venous tortuosity index. A P value of <0.05 was considered statistically significant.

Results

All patients were followed for at least 6 months after the initial injection of IVB or IVR. IVB was performed on 15 eyes and IVR on 16 eyes. The injections were administered two to five times (3.52 ± 0.88 (mean ± SD)) to each patient during the 6 months. According to the FA findings, the diagnosis of nonischemic CRVO was made in 26 eyes (Figs. 4 and 5), and the diagnosis of ischemic CRVO was made in 5 eyes. The SBP, DBP, heart rate, IOP, MAP, and MOPP were not significantly different among the baseline and 1, 3, and 6 months after the treatments ($P = 0.930$, $P = 0.958$, $P = 0.966$, $P = 0.745$, $P = 0.969$, $P = 0.886$, respectively).

At the baseline, the corrected MBR was 0.63 ± 0.26. The arteriovenous passage time on FA was 14.0 ± 5.2 s, and the venous tortuosity index on color fundus photograph was 1.113 ± 0.053. The corrected MBR was significantly correlated with the arteriovenous passage time ($r = -0.807$, $P < 0.001$; Fig. 6a) and venous tortuosity index ($r = -0.716$, $P < 0.001$). The corrected MBR after the first intravitreal injection was 0.60 ± 0.23 at 1 month, 0.61 ± 0.23 at 3 months, and 0.62 ± 0.28 at 6 months (Additional file 1). No significant differences were observed in the corrected MBR among the baseline and post-treatment times ($P = 0.379$). The MV in the affected eye was 34.8 ± 11.2 at the baseline, 32.0 ± 10.3 at 1 month, 31.6 ± 10.9 at 3 months, and 32.9 ± 12.2 at 6 months. No significant differences were observed in the MV among the baseline and post-treatment times ($P = 0.116$). The MT was 14.3 ± 4.6 at the baseline, 12.4 ± 3.8 at 1 month, 11.7 ± 4.0 at 3 months, and 12.3 ± 4.2 at 6 months. Significant differences were observed in the MT among the baseline and post-treatment times ($P = 0.003$). The MT at 1 and 3 months was significantly smaller than that at the baseline ($P = 0.011$, $P = 0.002$, respectively).

The mean BCVA was 0.68 ± 0.48 logMAR units at the baseline, 0.39 ± 0.36 logMAR units at 1 month after the first intravitreal injection, 0.36 ± 0.35 logMAR units at 3 months, and 0.33 ± 0.42 logMAR units at 6 months. Significant differences were observed in the BCVA among the baseline and post-treatment times ($P < 0.001$). The BCVA at 1, 3, and 6 months was significantly better than that at the baseline ($P < 0.001$, $P = 0.001$, $P = 0.007$, respectively).

The retinal sensitivity was 15.2 ± 7.5 dB at the baseline, 18.7 ± 6.6 dB at 1 month, 20.9 ± 6.0 dB at 3 months, and 20.4 ± 6.0 dB at 6 months. Significant differences were observed in the retinal sensitivity among the baseline and post-treatment times ($P < 0.001$). The retinal sensitivity at 1, 3, and 6 months was significantly better than that at the baseline ($P = 0.001$, $P < 0.001$, $P = 0.001$, respectively).

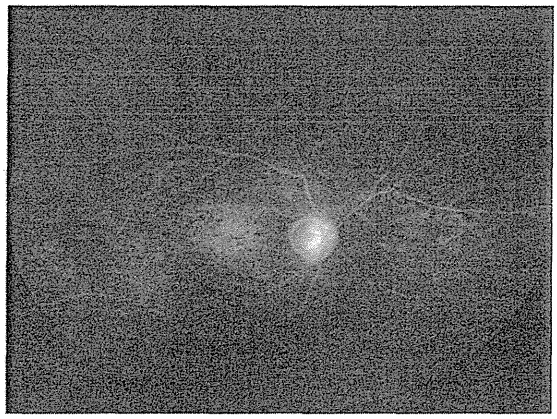

Fig. 4 Fluorescein angiography before anti-VEGF therapy for central retinal vein occlusion (CRVO). The same case presented in Fig. 1. Because there are not more than 10 disc areas of the nonperfusion areas, this case was diagnosed with nonischemic CRVO

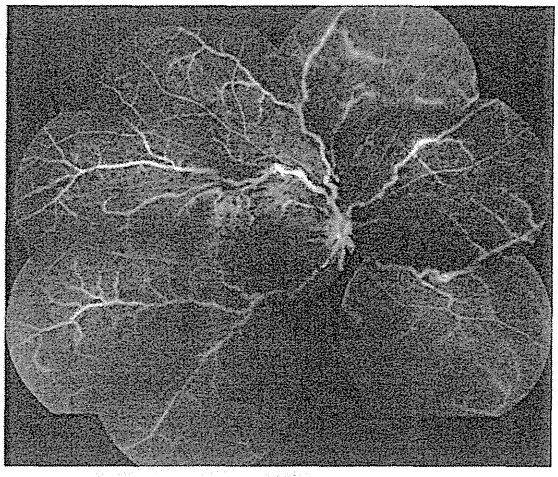

Fig. 5 Fluorescein angiography before anti-VEGF therapy for central retinal vein occlusion (CRVO). The same case presented in Fig. 2. Because there are not more than 10 disc areas of the nonperfusion areas, this case was also diagnosed with nonischemic CRVO

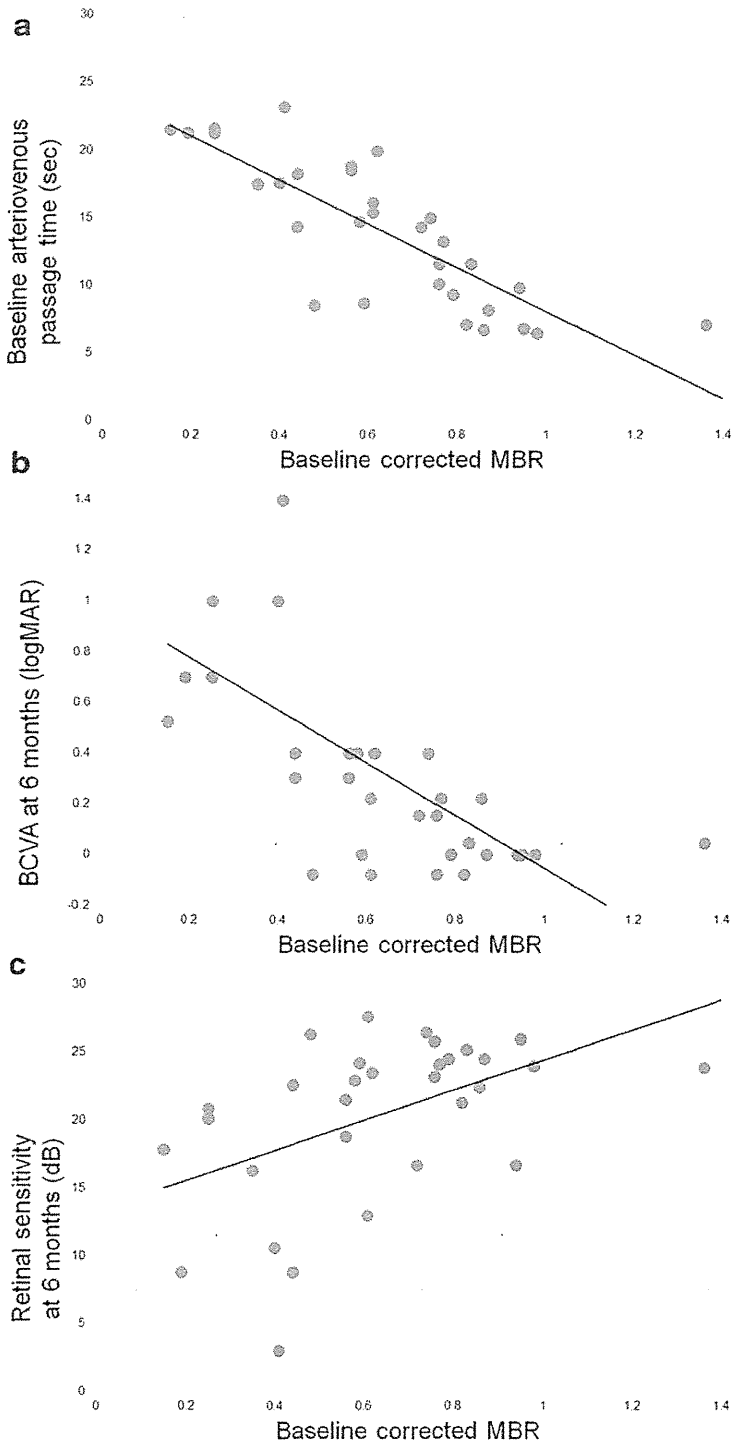

Fig. 6 (See legend on next page.)

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Fig. 6 Correlation between optic nerve head (ONH) circulation and arteriovenous passage time or visual function. Correlation between the baseline mean blur rate (MBR) at the ONH and the baseline arteriovenous passage time, the best-corrected visual acuity (BCVA) at 6 months, or mean retinal sensitivity at 6 months is presented. To exclude the influence of systemic circulation and blood flow of ONH tissue, the corrected MBR was calculated as; (MBR of ONH vessel area – MBR of ONH tissue area) in the affected eye ÷ (vascular MBR – tissue MBR) in the healthy fellow eye [11]. **a**: Correlation between the baseline corrected MBR at the ONH and the arteriovenous passage time at the baseline. The corrected MBR is significantly correlated with the arteriovenous passage time on fluorescein angiography ($r = -0.807$, $P < 0.001$). The solid line represents the linear regression line ($y = -16.102x + 24.200$). **b**: Correlation between the baseline corrected MBR at the ONH and BCVA in logMAR units at 6 months after the initial treatment. The corrected MBR is significantly correlated with the BCVA at 6 months ($r = -0.651$, $P < 0.001$). The solid line represents the linear regression line ($y = -1.036x + 0.983$). **c**: Correlation between the baseline corrected MBR at the ONH and mean retinal sensitivity at 6 months. The corrected MBR is significantly correlated with the retinal sensitivity at 6 months ($r = 0.485$, $P = 0.005$). The solid line represents the linear regression line ($y = 11.059x + 13.378$)

The MBR was significantly correlated with the BCVA (all $P < 0.005$) and the retinal sensitivity (all $P < 0.050$, Table 1) at all time points.

There was a significant correlation between the corrected MBR at the baseline and the BCVA at 6 months ($r = -0.651$, $P < 0.001$; Fig. 6b). There was also a significant correlation between the corrected MBR at the baseline and the retinal sensitivity at 6 months ($r = 0.485$, $P = 0.005$; Fig. 6c).

Discussion

The results showed that the corrected MBR at the baseline was significantly correlated with the BCVA and retinal sensitivity at 6 months after anti-VEGF therapy. This indicates that the visual prognosis is good in patients with CRVO who have good blood flow on the ONH at the baseline. In a report on 3 cases with CRVO, Matsumoto et al. also suggested that the prognosis of CRVO may be predicted by measuring the MBR using LSFG [13]. In eyes with ME due to a BRVO, Nagaoka et al. reported that the visual prognosis was good when the retinal blood flow measured by laser Doppler velocimetry before IVB was good [12]. Taken together, these results suggest that the status of the blood flow prior to the treatment is a useful predictor of the post-treatment BCVA for both CRVO and BRVO.

Table 1 Correlations between MBR on ONH and BCVA or retinal sensitivity before and after anti-VEGF therapy

	Corrected MBR on ONH			
	Baseline	1 month	3 months	6 months
BCVA (logMAR)	$r = -0.543$	$r = -0.672$	$r = -0.546$	$r = -0.565$
	$P = 0.001$	$P < 0.001$	$P = 0.001$	$P < 0.001$
Retinal sensitivity (dB)	$r = 0.572$	$r = 0.608$	$r = 0.552$	$r = 0.430$
	$P < 0.001$	$P < 0.001$	$P = 0.001$	$P = 0.015$

BCVA, best corrected visual acuity; Corrected MBR, (MBR of ONH vessel area – MBR of ONH tissue area) in the affected eye ÷ (vascular MBR – tissue MBR) in the unaffected eye; logMAR, logarithm of the minimum angle of resolution; MBR, mean blur rate; ONH, optic nerve head; VEGF, vascular endothelial growth factor

The measurements of the LSFG had excellent reproducibility which then permitted a non-invasive method to measure the ocular circulation. Aizawa et al. used LSFG and reported that the intra-session reproducibility of the MBR in the ONH of three continuous examinations was excellent with a coefficient of variation of 3.4 ± 2.0 and an intraclass correlation coefficient of 0.95 [9]. Aizawa et al. also reported that the MBR of the ONH tissue was strongly correlated with the visual field sensitivity in eyes with glaucoma [3]. In addition, Maekubo et al. reported that measurements of the ONH circulation by LSFG could be used for differentiating nonarteritic ischemic optic neuropathy from anterior optic neuritis [19]. Based on these findings, measuring the ONH circulation by LSFG can be considered a useful method in the clinic.

Yamada et al. measured the MBR on the ONH in eyes with untreated CRVO [11]. The corrected MBR was calculated to exclude the influence of the systemic circulation and blood flow of the ONH tissue. After obtaining the MBR values by subtracting the MT from the MV, the ONH blood flow was evaluated by taking the MBR values of the affected eye divided by the MBR values of the healthy fellow eye. The authors reported that this corrected MBR was significantly correlated with the arteriovenous passage time [11]. Similarly, a significant correlation was found between the corrected MBR and arteriovenous passage time in the present study. The strong correlation between the corrected MBR and arteriovenous passage time, which has been used as an indicator of retinal circulation, confirms that the method we used is a highly reliable way to evaluate the circulatory status of the ONH. In addition, Yamada et al. reported that the corrected MBR values in large ONH vessels were significantly correlated with the aqueous VEGF concentrations in eyes with CRVO [11].

It is sometimes difficult to distinguish the vessel and tissue areas at the ONH in eyes with CRVO, because dilatation or blood stasis of capillary vessel on ONH obscures the boundary between the large vessel and tissue

on the LSFG images. Thus, the MV and MT values measured in eyes with CRVO may include a measurement error. However, the corrected MBR in the present study was significantly correlated with not only arteriovenous passage time on FA but also the venous tortuosity index on fundus photograph, which suggests that this measurement error is considered to be small.

Nitta et al. reported that there was no significant change in the MBR measured by LSFG in the retinal artery, retinal vein, or ONH after a single IVB injection in eyes with a BRVO [22]. Nagaoka et al. also reported no significant changes in the retinal blood flow measured by laser Doppler velocimetry after a single IVB injection for BRVO [12]. Consistent with these findings, our results showed no significant differences in the corrected MBR after IVB or IVR in eyes with a CRVO. On the other hand, Matsumoto et al. reported 3 cases of ME secondary to nonischemic CRVO in which the blood flow increased after IVBs [13]. In their case, the MBR increased in 3 or 4 weeks after the IVB was confirmed by LSFG, but long term changes of the MBR and exact relationship between the MBR and visual prognosis were unclear.

This study has several limitations. First, the sample size was small, and the follow-up period was short. Further studies with a larger sample size and longer follow-up periods would be required to confirm our findings. We also need to keep in mind that when using LSFG the MBR is a relative value of the blood flow velocity and not the absolute value. Therefore, when comparing the difference between eyes, we used the corrected MBR value. The other limitation is that the present study included both patients treated with IVB and those with IVR. Lastly, Mayama et al. reported that phenylephrine eye drops can depress the ONH circulation [23]. In our study, Mydrin P, tropicamide and phenylephrine, was used prior to the LSFG testing to obtain more accurate measurements of the ONH circulation with LSFG. Thus, there is a possibility that phenylephrine may have influenced the ONH circulation. However, because Mydrin P was administered to both the affected and unaffected eyes and ONH circulation was evaluated using the equation; the MBR of the affected eye/MBR of the unaffected eye, the magnitude of this influence may be small.

Conclusions

The blood flow velocity on the ONH was significantly correlated with the BCVA and retinal sensitivity before and after anti-VEGF therapy for CRVO. We suggest that the blood flow velocity of the ONH before the therapy is a predictive factor for the visual outcome after treatment for CRVO.

Ethics and consent to participate

Approval was obtained from the Institutional Review Board of Saneikai Tsukazaki Hospital prior to beginning this study, and the patients gave their written informed consent prior to their inclusion. The procedures used in this study adhered to the tenets of the Declaration of Helsinki.

Consent to publish

The patients have provided permission to publish clinical data of their case in this study.

Additional file

Additional file 1: Raw data (MBR, visual function). (DOC 68 kb)

Abbreviations

asb: apostilb; BCVA: best-corrected visual acuity; BRVO: branch retinal vein occlusion; CRVO: central retinal vein occlusion; DBP: diastolic blood pressure; FA: fluorescein angiography; IOP: intraocular pressure; IVB: intravitreal bevacizumab; IVR: intravitreal ranibizumab; LSFG: laser speckle flowgraphy; MAIA: macular integrity assessment; MAP: mean arterial pressure; MBR: mean blur rate; ME: macular edema; MOPP: mean ocular perfusion pressure; MT: mean blur rate of tissue area; MV: mean blur rate of vessel area; OCT: optical coherence tomography; ONH: optic nerve head; SBP: systolic blood pressure; SD-OCT: spectral-domain optical coherence tomography; UMIN: University hospital Medical Information Network; VEGF: vascular endothelial growth factor.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Design of the study (YM), conduct of the study (DN, TN, YY, HT), management of the data (DN, YM, KS, KA, TN, YY, HT), analysis of the data (DN, YM, KS, KA, YK), interpretation of the data (DN, YM, KS, KA, YK), preparation of the manuscript (DN, YM) and overall coordination (YK). All authors read and approved the final manuscript.

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Author details

[1]Department of Ophthalmology, Saneikai Tsukazaki Hospital, Himeji, Japan. [2]Department of Ophthalmology and Visual Sciences, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan. [3]Department of Ophthalmology, Institute of Biomedical Sciences, Tokushima University Graduate School, 3-18-15 Kuramoto, Tokushima 770-8503, Japan.

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LABORATORY INVESTIGATION

Effects of topical adrenergic agents on prostaglandin E2-induced aqueous flare and intraocular pressure elevation in pigmented rabbits

Momoko Nakamura-Shibasaki[1] · Miftahul Akhyar Latief[2] · Ji-ae Ko[1] · Kunihiko Funaishi[1] · Yoshiaki Kiuchi[1]

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Abstract

Purpose To evaluate the effects of signals through adrenergic receptors on the changes in the aqueous flare and intraocular pressure (IOP) induced by topical prostaglandin E2 (PGE2) in pigmented rabbits.

Methods Adrenergic agents were applied topically to pigmented Dutch rabbits, and PGE2 was then applied to induce an increase in the aqueous flare and IOP. The degree of aqueous flare was measured with a laser flare meter, and the IOP was measured with a rebound tonometer. Measurements were made every 30 min after the PGE2 had been applied for 2 h and at 4.0 and 4.5 h. Repeated measure analysis of variance and Dunnett's post hoc tests were used for the statistical analyses.

Results The topical application of PGE-2 increased the aqueous flare for more than 4.5 h. The topical instillation of 1.0 % apraclonidine significantly inhibited the increase in the PGE2-induced aqueous flare by 75.1 %, of 0.1 % brimonidine by 57.2 %, of 0.04 % dipivefrin by 57.4 %, and a combination of 0.1 % brimonidine and 5 % phenylephrine by 78.9 %. Topical 5.0 % phenylephrine and 0.05 % isoproterenol had little effect on the aqueous flare elevation induced by PGE2. The IOP increased 0.5 h after the topical application of PGE-2. Topical 1.0 % apraclonidine, 0.1 % brimonidine, 0.1 % dipivefrin, and the combination of 0.1 % brimonidine and 5.0 % phenylephrine significantly inhibited the PGE2-induced IOP elevation. However, topical 5.0 % phenylephrine and 0.05 % isoproterenol did not significantly inhibit the IOP elevation caused by PGE2.

Conclusions Signaling by the α2 receptor inhibits both the PGE2-induced flare and IOP elevation caused by topical PGE2 application.

Keywords Adrenergic agents · Prostaglandin E2 · Aqueous flare · Intraocular pressure

Introduction

Adrenergic agents alter the aqueous humor dynamics and intraocular pressure (IOP) of the eye and are often used as IOP-lowering agents [1]. Epinephrine used to be administered worldwide as a standard topical drug for the management of chronic open-angle glaucoma. This direct-acting sympathomimetic agent stimulates all α- and β-adrenergic receptors. Within minutes after the instillation of epinephrine, the aqueous inflow is reduced, most likely because of α-adrenergic vasoconstriction. The vasoconstriction then reduces the ultrafiltration of plasma into the stroma of the ciliary process and leads to an increase in outflow facility [1, 2]. The mechanism underlying this improved outflow has not been definitively determined. Apraclonidine hydrochloride is a para-amino acid derivative of clonidine hydrochloride and is classified as an α2-adrenergic agonist. Apraclonidine lowers the IOP by reducing the aqueous production and increasing the uveoscleral aqueous humor outflow [3, 4]. Brimonidine tartrate is a highly selective α2-agonist that lowers the IOP by reducing the production of aqueous humor [5–7].

✉ Yoshiaki Kiuchi
ykiuchi@hiroshima-u.ac.jp

[1] Department of Ophthalmology and Visual Sciences, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minamiku, Hiroshima 734-8551, Japan

[2] Muhammadiyah University, Sultan Alauddin Street No. 259, Makassar City 90221, South Sulawesi, Indonesia

Adrenergic agents are also known to have antiinflammatory effects. The β2 stimulants are used to treat bronchial asthma because of their bronchodilatory and antiinflammatory effects [8]. In dermatology, isoproterenol, a β stimulant, is known to prevent the release of histamine from mast cells [9]. The application of isoproterenol to retinal Muller cells in culture decreases the expression of iNOS, TNF-α, IL-1β and PGE2 and is also believed to suppress the production of inflammatory cytokines [10].

Although apraclonidine is known to reduce the IOP and inflammation [11–14], no studies have examined whether brimonidine, an α2-adrenergic agonist, has any antiinflammatory effects. Conversely, several reports show that it can cause treatment-associated uveitis. Several early studies report patients who had developed granulomatous uveitis during brimonidine treatment [15–18]. The differences in the effect of apraclonidine and brimonidine on the degree of inflammation and IOP increase induced by inflammation have not been fully examined. In addition, the effects of β or α1 agonists on flare intensity and IOP changes in eyes with inflammation have also not been extensively examined.

Camras et al. [19] topically applied 25–200 μg of PGE2 and PGF2 α to rabbit eyes, and they found a prolonged (15–20 h) ocular hypotony following the initial hypertensive phase. The aqueous humor protein concentration was increased 30- to 50-fold 2 h after the administration of 50 μg PGE2. Hayasaka et al. [14, 20] also found the application of PGE2 to the eye can cause inflammation in a relatively short time [14, 19, 20]. They studied the effect of several medicines on the eye with inflammation and proved that the application of PGE2 to the eye could be a good experimental model to examine the effect of the medication on inflammatory eyes [14, 19, 20]. The elevation in the aqueous flare was reproducible when PGE2 was reapplied more than 1 week later [14, 20].

The purpose of this study was to evaluate the effects of topical instillation of an α1 agonist (phenylephrine), α2 agonists (apraclonidine and brimonidine), β agonist (isoproterenol) and one α β agonist (dipivefrin, a pro-drug of epinephrine) on the aqueous flare and IOP elevation induced by PGE2 in pigmented rabbits.

Materials and methods

Animals

The animals were housed and treated according to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. The procedures used were performed in accordance with the guidelines of the Committee on Animal Experimentation and the Committee of Research Facilities for Laboratory Animal Science, Natural Science Center for Basic Research and Development of Hiroshima University. Six male pigmented rabbits (2.5–3.0 kg, Charles River Japan, Yokohama, Japan) were used for the study.

Chemicals

Dipivefrin hydrochloride 0.1 %, a pro-drug of epinephrine (an α and β stimulant), was obtained from Santen Pharmaceutical Co. (Osaka, Japan). The 1.0 % apraclonidine hydrochloride, an α2 agonist, 0.1 % brimonidine tartrate, an α2 agonist, and 5.0 % phenylephrine hydrochloride, an α1 agonist, were purchased from Alcon Laboratories (Fort Worth, TX, USA), Senju (Osaka, Japan) and Kowa (Tokyo, Japan), respectively.

All commercially available eye drops were applied to the rabbits' eyes without changing the formulation of the eye drops. Because there is no commercially available form of isoproterenol eye drops, we purchased isoproterenol from Sigma Chemical Co. (St. Louis, MO, USA) and diluted it with PBS in accordance with a previous report, adjusting it to a concentration to 0.05 % [19]. PGE2 was obtained from Funakoshi Chemicals (Tokyo, Japan), dissolved in 100 % ethanol and stored at −70 °C. The PGE2 solution was diluted to 5.0 % in ethanol and with 0.9 % NaCl immediately before use [14].

Topical instillation of adrenergic agents and placebo

Fifty microliters of the adrenergic agents or 0.9 % NaCl (placebo) was topically instilled in the left eyes of the rabbits. Thirty minutes later, 50 μl of PGE2 (2.5 μg) was instilled into the eye. When applying the combination of brimonidine and phenylephrine, the phenylephrine was applied 5 min after the application of brimonidine.

Aqueous flare measurement and tonometry

The degree of aqueous flare was measured with a laser flare meter (FM600: Kowa, Tokyo, Japan) according to the method described by Sawa et al. [21]. The laser flare meter can evaluate the level of intracameral proteins noninvasively. Five measurements were obtained at each time point, and the mean values were used for the statistical analyses. The measurements were made at about the center of the anterior chamber in a 0.3 × 0.5-mm area.

The IOP was measured with a rebound tonometer (RBT; Tiolat Oy, Helsinki, Finland) according to the instruction manual, i.e., 6 mm from the center of the cornea with the probe perpendicular to the corneal plane. The IOP was measured just after measuring the anterior aqueous flare. We converted the IOP readings of the rebound tonometer to the real IOP value using the equation obtained in preliminary experiments:

True IOP = 1.63 × (rebound tonometer value) + 9.35.

In preliminary experiments, the transducer was connected to a BSS bottle the height of which above the transducer was changed. The reliability of the transducer was confirmed within a range of 5–50 mmHg ($r^2 = 0.99$, $P < 0.0001$). Then, the BSS reservoir was connected to the rabbit's anterior chamber, and the height was sequentially reduced from 50–5 mmHg in steps of approximately 5 mmHg. The IOP was measured with the RBT at each level.

Only the left eyes were used for the experiments. The aqueous flare and IOP measurements were obtained every 30 min for 2 h and at 4 and 4.5 h after the topical application of the ocular hypotensive agents or saline. The interval between the experimental sessions on one eye was 1–2 weeks (Fig. 1).

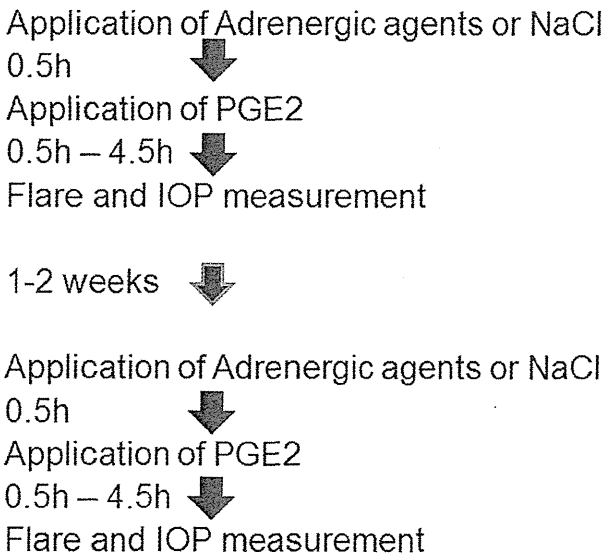

Fig. 1 The time course of the experiment

Statistical analyses

The obtained data were analyzed using repeated-measures analysis of variance (ANOVA) to assess the time course of changes in the aqueous flare and IOP over 4½ h after the instillation of PGE2. The degree of aqueous flare increase was expressed as the area under the curve (AUC) for each eye. Subjects who received 0.9 % NaCl served as controls. The significance of the AUC of the flare was evaluated using one-way ANOVA with repeated measurements and Dunnett's post hoc test.

The IOP was compared among the drugs at each time point using one-way ANOVA with repeated measurements. Post-hoc Dunnett mean comparison tests were performed to evaluate the differences between the control (0.9 % NaCl) and adrenergic agents if one-way ANOVA was significant. All data are expressed as the means ± standard deviations.

A value of $P < 0.05$ for one-way ANOVA with repeated measurements and <0.0071 for the post hoc Dunnett test was considered statistically significant. The statistical analyses were performed using the JMP software program, version 9.0 (SAS Institute, Inc. Cary, NC, USA).

Results

Aqueous flare

After the PGE2 had been applied in the control group, the degree of aqueous flare increased, reaching a maximum of 149.5 ± 74.2 photon counts/ms at 2 h. Thereafter, it gradually decreased to 92.9 ± 53.4 photon counts/ms at 4½ h (Fig. 2). None of the adrenergic agents was able to

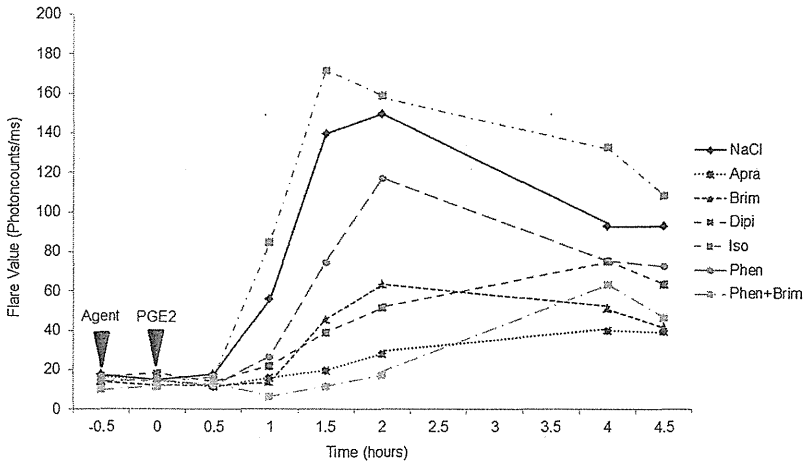

Fig. 2 The time courses of the change in the anterior chamber flare. Fifty microliters of the adrenergic agents or 0.9 % NaCl (placebo) was topically instilled in the left eyes at time −0.5 h. Thirty minutes later, 50 µl PGE2 (2.5 µg) was instilled into the eye

Table 1 Flare changes due to instillation of agents followed by PGE-2

Agents	Time course (h)							*P*
	0 h	0.5 h	1 h	1.5 h	2 h	4 h	4.5 h	
NaCl	15.3 ± 12.2	17.9 ± 8.7	55.8 ± 8.9	139.7 ± 64.8	149.5 ± 74.2	92.6 ± 46.5	92.9 ± 53.4	<0.0001*
Apra	15.0 ± 1.5	11.5 ± 3.6	16.0 ± 8.9	19.7 ± 5.8	28.2 ± 10.9	39.9 ± 32.6	39.3 ± 24.3	0.0168*
Brim	12.1 ± 3.6	12.4 ± 3.0	13.9 ± 3.6	45.7 ± 35.2	63.5 ± 54.8	51.1 ± 35.6	41.7 ± 28.8	0.0188*
Dipi	18.3 ± 7.9	14.4 ± 3.5	22.0 ± 5.7	39.1 ± 29.5	51.5 ± 40.1	75.3 ± 32.6	63.6 ± 26.8	0.0005*
Iso	14.4 ± 6.2	16.4 ± 7.7	84.7 ± 14.1	171.7 ± 50.1	159.2 ± 34.8	132.8 ± 78.4	108.5 ± 55.6	<0.0001*
Phen	15.1 ± 8.1	12.5 ± 7.1	26.8 ± 9.6	74.9 ± 34.5	117.4 ± 74.3	75.4 ± 49.3	73.0 ± 43.0	0.0003*
Phen + Brim	11.7 ± 2.5	13.5 ± 3.4	6.5 ± 3.3	11.8 ± 5.4	17.5 ± 4.9	63.7 ± 56.8	46.6 ± 47.8	0.0074*

The data are expressed as the mean ± SD. The number of rabbits was 6

NaCl NaCl 0.9 %; *Apra* apraclonidine 1.0 %; *Brim* brimonidine 0.1 %; *Dipi* dipivefrine 0.1 %; *Iso* isoproterenol 0.05 %; *Phen* phenirephrine 5 %; *Brim + Phen* combination of brimonidine 0.1 % and phenirephrine 5 %

* Significant difference using one-way ANOVA with repeated measurement ($P < 0.05$)

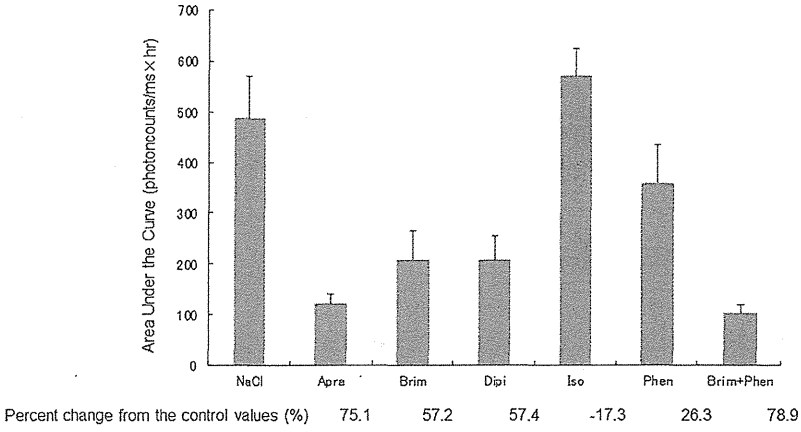

Fig. 3 The Area under the *curve* of the flare intensity and percent change of AUC from the control. The degree of aqueous flare increase was expressed as the area under the *curve* for each eye. Subjects who received 0.9 % NaCl served as controls

suppress the flare changes caused by PGE2 application (Fig. 2; Table 1).

The area under the curve of the aqueous flare intensity after the application of adrenergic agents and PGE2 is shown in Fig. 3 and Table 2. One-way ANOVA with repeated measurement showed that the AUCs in each group were not identical ($P < 0.0001$). The instillation of 1.0 % apraclonidine hydrochloride, inhibited the aqueous flare elevation by 75.1 % ($P = 0.0003$), of 0.1 % brimonidine tartrate by 57.2 % ($P = 0.0064$) and of 0.1 % dipivefrin hydrochloride by 57.4 % ($P = 0.0062$) of the PGE2-induced increase in aqueous flare. The degree of inhibition induced by the combination of 5.0 % phenylephrine hydrochloride and 0.1 % brimonidine tartrate was 78.9 % ($P = 0.0001$), approximately the same as that induced by 1.0 % apraclonidine hydrochloride. One instillation of 5.0 % phenylephrine hydrochloride eye drops inhibited the PGE2-induced increase in the aqueous flare by 26.3 %. However, the difference in the degree of inhibition was not significant ($P = 0.4028$). Topical

Table 2 Comparison of area under the curve of flare changes among the agents

	Flare (AUC)	*P* (compaired with control)
Control (NaCl)	487.3 ± 203.4	–
Apra	121.3 ± 44.4	0.0003*
Brim	208.4 ± 142.1	0.0064*
Dipi	207.5 ± 115.1	0.0062*
Iso	571.4 ± 133.9	0.7823
Phen	358.8 ± 189.0	0.4028
Brim + Phen	102.6 ± 41.9	0.0001*

The AUCs are expressed as the mean ± SD. The number of rabbits was 6

NaCl NaCl 0.9 %; *Apra* apraclonidine 1.15 %; *Brim* brimonidine 0.1 %; *Dipi* dipivefrine 0.1 %; *Iso* isoproterenol 0.05 %; *Phen* phenirephrine 5 %; *Brim + Phen* combination of brimonidine 0.1 % and phenirephrine 5 %

* Significant difference using Dunnett mean comparison test ($P < 0.0071$)