complicated by cardiac and respiratory insufficiency requiring ventilation assistance. He had no family history of ocular disease or trauma, and his best-corrected visual acuity was 0.01 in the right eye and hand movements in the left eye. The intraocular pressure was not measured. The anterior segment examination with a handheld slit lamp was unremarkable, except for mild cataracts in both eyes. Ophthalmoscopy showed proliferative tissue on the disks and condensed vitreous hemorrhage around the posterior poles bilaterally, but the peripheral retina appeared to be attached
F1 (Figure 1). Ultrasound echography showed a tent-shaped vitreous cortex that was attached to the posterior retina in the right eye. The
F2 posterior retina was also detached in the left eye (Figure 2).

The single-flash full-field electroretinogram consisted of a reduced *b* wave with preserved *a* wave. The amplitude of the *a* wave was larger than that of the *b* wave in the right eye, making a negative-type electroretinogram. An electroretinogram was not recorded from the left eye (Figure 2).

Blood tests showed 2.56×10^6 μL of erythrocytes, 8.2 g/dL of hemoglobin, 60.8 mg/dL of blood urea nitrogen, and 0.4 mg/dL of creatinine. The leukocytes and C-reactive protein were within the normal range. The cardiac ejection fraction was 52% of the predicted level. The patient had no history of diabetes mellitus or hypertension. Because of the cardiac and respiratory insufficiency, we recommended vitreous surgery on the right eye under local anesthesia, and permission was obtained from the patient and family for the surgical procedures. Pars plana vitrectomy and pars plana lensectomy combined with laser photocoagulation and silicone oil injection were successfully performed on the right eye in December. After 3 months, the silicon oil was removed and an intraocular lens was implanted. The best-corrected visual acuity improved to 0.8 in the right eye, and this vision was maintained for 12 months. The right fundus photograph taken 1 year after the last
F3 surgical procedure is shown in Figure 3. The primary ophthalmologist, who referred this patient, performed pars plana vitrectomy on the left eye under local anesthesia 1 month after the first surgery on the right eye but failed to reattach the retina. The best-corrected visual acuity remained at hand movements in the left eye.

Discussion

The mutation causing DMD is the gene encoding dystrophin, a 427-kDa protein expressed in photoreceptor terminals and around retinal vessels.[2,3] Most DMD patients have reduced *b*-wave amplitudes with preserved *a* wave under scotopic conditions.[4] Thus, the electroretinogram has the negative-type pattern, as was found in our patient and reported for other patients with DMD. It is believed that the deficiency of dystrophin leads to abnormal transmission between the photoreceptors and subsequent neural cells resulting in the reduced *b* waves.[5]

The pathogenesis of the severe proliferative retinopathy with neovascularization is unknown. A search of PubMed extracted only two studies that reported on the association of proliferative retinopathy and DMD.[7,8] Louie et al[7] stated that they believed that cardiopulmonary compromise was the primary contributor to the development of retinal neovascularization. Ober et al[8] presented a case of DMD with cardiomyopathy and anemia. They hypothesized that their patient with chronic anemia and cardiovascular compromise had surpassed the threshold beyond which retinal neovascularization might be stimulated. Neither cardiopulmonary compromise nor the absence of dystrophin usually leads to retinal neovascularization. The location of dystrophin around retinal vessels of DMD patients suggests that they may be more susceptible to retinal hypoxemia, which can then lead to retinal neovascularization. Because we examined only one patient, we cannot generalize our finding of retinal neovascularization in patients with DMD. However, clinician should be aware that proliferative retinopathy can be associated with retinal vascular complications in patients with DMD. All three cases reported to date, our case and the two cases from the literature,[7,8] involved patients in the very late stages of DMD, and an early surgery may have improved the success rate.

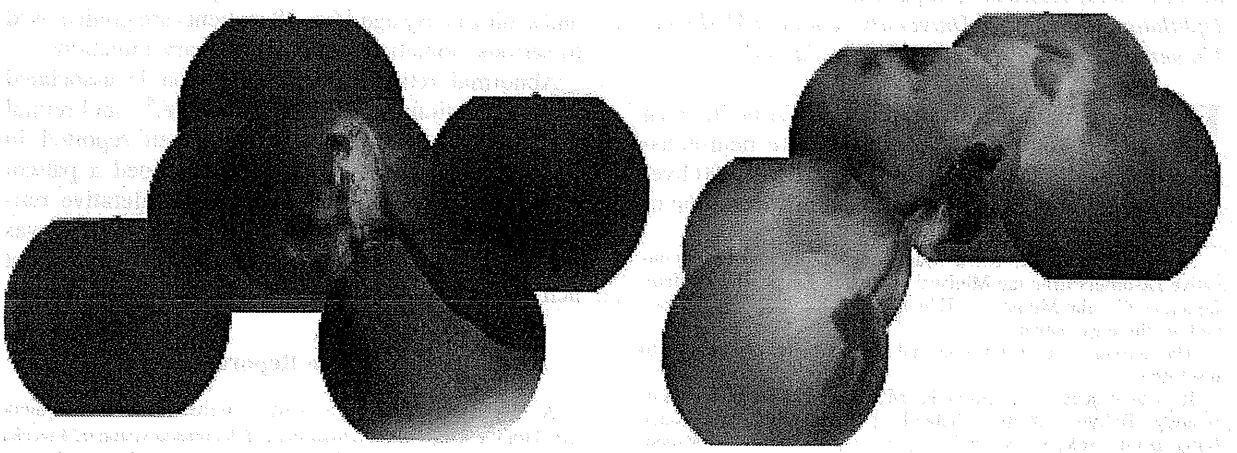

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Fig. 1. Fundus photograph of the right (left) and left (right) eyes showing that the optic disks and maculas are partially obscured by hemorrhage.

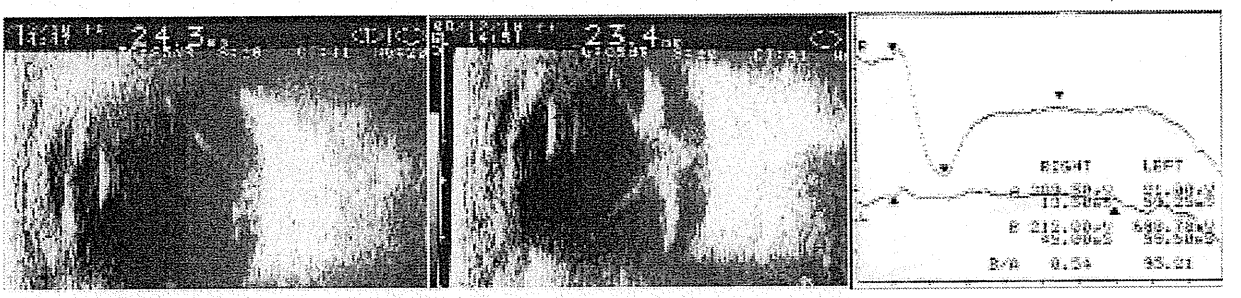

Fig. 2. Ultrasound echograms of the right (left) and left (middle) eyes showing vitreoretinal adhesions in the posterior pole. In the left eye, proliferative tissue and tractional retinal detachment can be seen. Single-flash electroretinogram (right) showing attenuated *b* wave and normal *a* wave resulting in a negative-type electroretinogram in the right eye and no response in the left eye.

Pars plana vitrectomy was effective in the right eye, whereas the vitreous hemorrhage and tractional retinal detachment prevented proper treatment such as photocoagulation or surgery on the left eye. Considering that an early treatment has the potential to improve the prognosis, periodic fundus examination is warranted in patients with DMD.

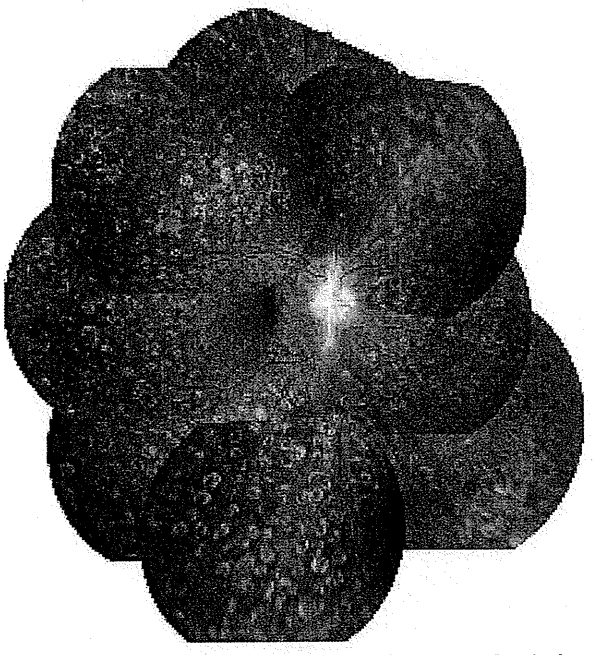

Fig. 3. Fundus photograph of the right eye taken 1 year after the last surgical procedure.

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The authors are deeply grateful to the late Professor Shinichiro Kawano who cared for the patient including the successful vitrectomy.

Key words: Duchenne muscular dystrophy, proliferative retinopathy, pars plana vitrectomy, dystrophin.

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SIMULTANEOUS BILATERAL CENTRAL RETINAL ARTERY OCCLUSION IN CHURG–STRAUSS SYNDROME

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Purpose: Retinal vascular abnormalities are rare in patients with Churg–Strauss syndrome. We present the findings in a patient with Churg–Strauss syndrome who developed bilateral central retinal artery occlusion simultaneously.

AQ : 1 **Methods:** Case report.

Results: A 68-year-old Japanese man developed acute bilateral vision decrease to counting finger in the right eye and hand movements in the left eye. Ophthalmoscopic and angiographic examinations revealed a central retinal artery occlusion with choroidal circulatory disturbances in both the eyes. The patient had bronchial asthma, hypereosinophilia, radiographically determined migratory pulmonary opacities, and paranasal sinus abnormalities, thus fulfilling the American College of Rheumatology criteria for Churg–Strauss syndrome. Antineutrophil cytoplasmic antibody was absent. High-dose steroid therapy was used, but after 6 weeks, his visual acuity in the right eye did not improve, and the vision in the left eye was no light perception. Later, vitreous hemorrhage was developed in the left eye followed by retinal detachment associated with proliferative retinopathy.

Conclusion: Bilateral central retinal artery occlusion can occur in patients with antineutrophil cytoplasmic antibody–negative Churg–Strauss syndrome. The cause of the central retinal artery occlusion is not known, but consideration for prophylactic steroid therapy may be recommended in antineutrophil cytoplasmic antibody–negative cases to prevent potential visual loss.

RETINAL CASES & BRIEF REPORTS X:1–5, 2011

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Churg–Strauss Syndrome (CSS) is an uncommon systemic disease characterized by asthma, hypereosinophilia, and vasculitis of different organs.[1] Ocular involvements are infrequent and include conjunctival nodules, panuveitis, orbital myositis, ischemic optic neuropathy, and retinal artery and vein occlusion.[2–5] There has recently been a rapidly growing literature concerning retinal vascular complications in CSS.[4–12] We present a patient with CSS who developed simultaneous bilateral central retinal artery occlusion (CRAO).

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Case Report

A 68-year-old Japanese man was seen by an internist for his regular appointment for chronic asthma in October 2008. Because his laboratory data showed marked elevations of leukocyte count (17,850 per mm^3) and eosinophils (10,000 per mm^3; 56.0%), he was referred and admitted in the department of internal medicine in our hospital. Neurologic examinations on admission were unremarkable. Laboratory data showed 17,000 per cubic millimeter of leukocytes with eosinophilia of 3.8×10^9 per liter (17%), an erythrocyte sedimentation rate of 42 mm at 1 hour, and a C-reactive protein of 7.27 mg/dL (reference value <0.5 mg/dL). The results from blood tests for renal, hepatic, and pancreatic function were normal. Urinalysis showed no abnormalities. Blood coagulation

tests were within normal limits. There was a marked elevation of rheumatoid factor 50.1 IU/mL (reference value <20 IU/mL). Tests for antinuclear antibodies, antineutrophil cytoplasmic antibodies (ANCA), the lupus anticoagulant, anticardiolipin antibodies, and circulating immune complexes were negative. The patient did not have a history of diabetes mellitus or hypertension. A computed tomography scan and a magnetic resonance imaging scan of the brain showed no abnormalities except for sinusitis. Radiographic examinations of the chest showed multiple migratory opacities in both bronchi.

An acute vision reduction was bilaterally developed in the patient that was thought to be CSS by the systemic signs and symptoms, and he was referred to the Ophthalmology Department. He had no history of ocular disease or trauma, and his best-corrected visual acuity was counting finger in the right eye and hand movement in the left eye. The intraocular pressure was 12 mmHg in both the eyes. The anterior segment examination was unremarkable except for mild cataracts in both the eyes.

Ophthalmoscopy showed marked retinal whitening, a cherry red spot, and attenuated retinal arteries without an embolus in both the eyes (Figure 1). Fluorescein angiography showed a marked delay in choroidal and retinal artery filling, and the arteriovenous transit time was increased. There were signs of possible reestablishment of the retinal circulation in both the eyes. There also appeared to be choroidal circulatory compromises in both the eyes with patchy filling delay of the choroidal vessels (Figure 1). F1

A diagnosis of CSS with the retinal vascular involvement was made. Intravenous methylprednisolone, 1 g/day, for 3 days was administrated twice. Thereafter, oral prednisolone was started from 60 mg/day and was gradually tapered and kept at 27.5 mg/day. The eosinophil count normalized together with a decline in the erythrocyte sedimentation rate and C-reactive protein. However, 4 weeks later, the visual acuity in the right eye had not improved and decreased to no light perception in the left eye. After 6 months, the left eye developed dense vitreous hemorrhage (Figure 2). The intraocular pressure was 16 mmHg in both the eyes, and rubeosis was not observed in both the eyes. Ophthalmoscopy showed a pale optic disk in the right eye and dense vitreous hemorrhage in the left eye. Ultrasound echography showed a detached retina and proliferative retinopathy in the left eye (Figure 2). F2

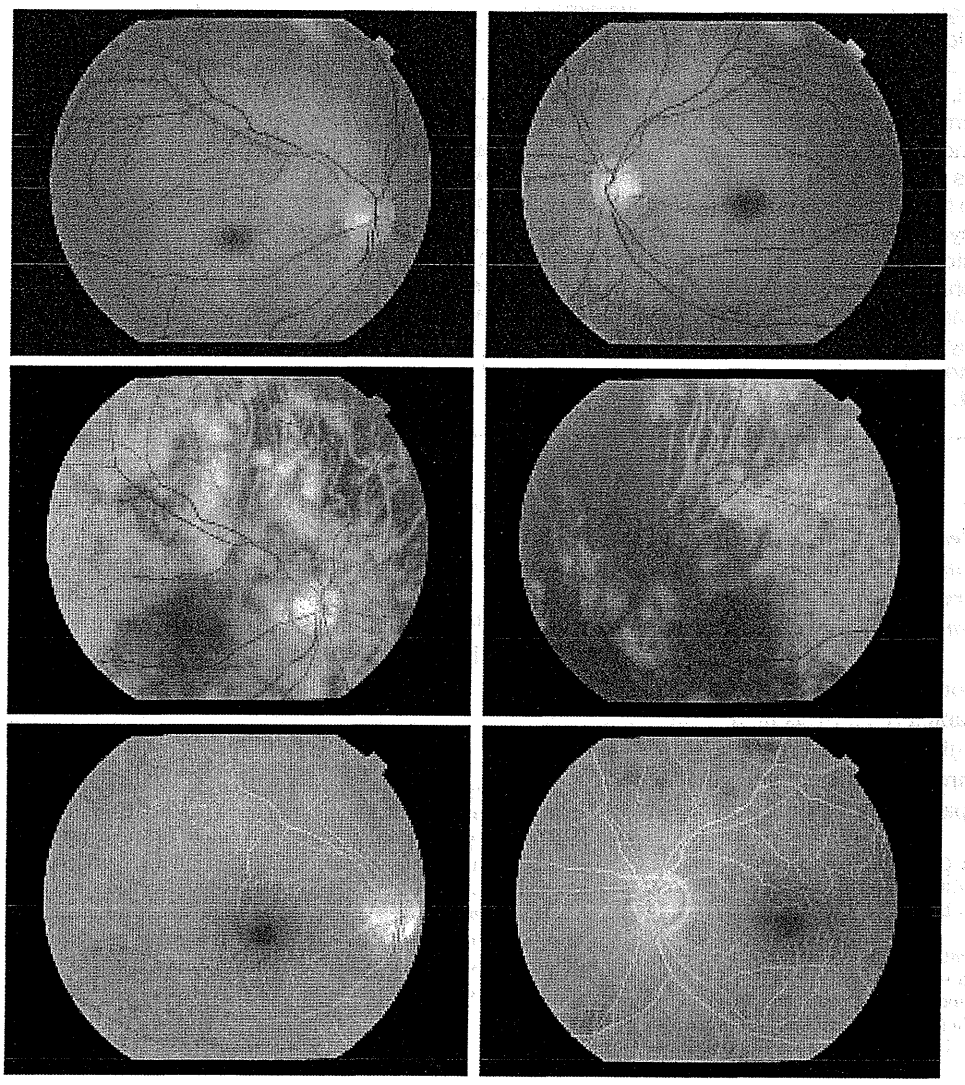

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Fig. 1. Fundus photographs and fluorescein angiograms on the day after the onset of central retinal artery occlusion. **A** and **B.** Fundus photographs showing cherry red spot in both the eyes. **C** and **D.** Fluorescein angiograms at the early phase showing marked delay in retinal artery filling and choroidal circulatory compromise in both the eyes. **E** and **F.** Fluorescein angiograms at the arteriovenous phase showing delayed arteriovenous transit time and possible reestablishment of the retinal circulation in both the eyes.

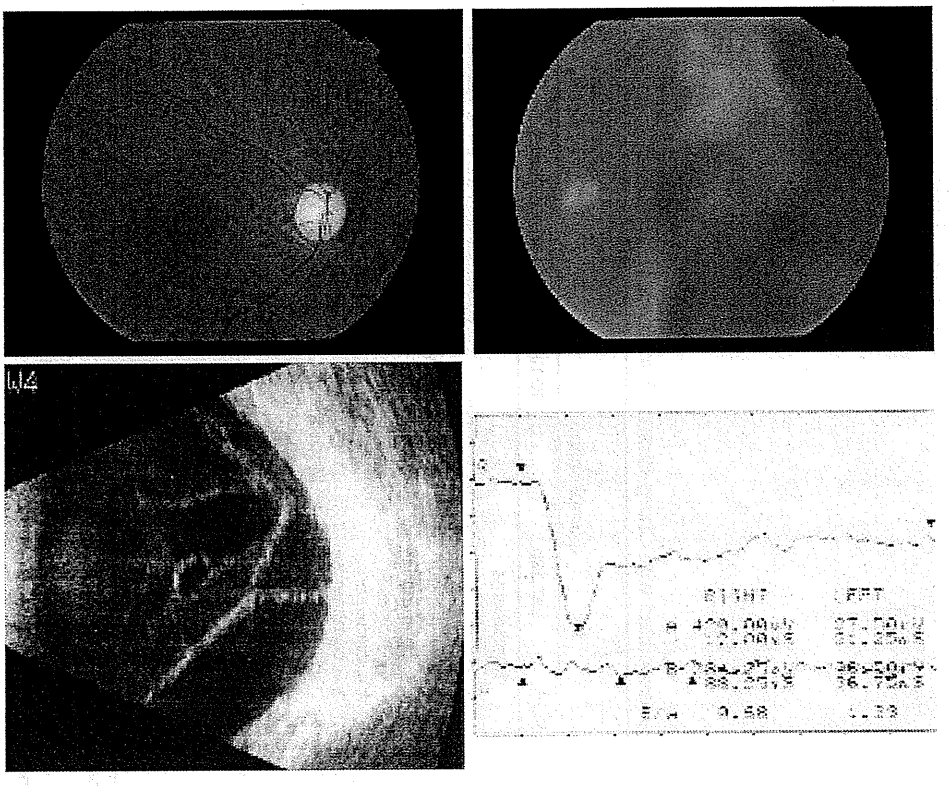

Fig. 2. Ultrasound echography and electroretinography 6 months after the onset. Left: A B-mode ultrasound photograph showing a detached retina in association with proliferative retinopathy in the left eye. Right: Single-flash, full-field electroretinogram consists of a reduced *b* wave with preservation of the *a* wave, leading to a negative-type electroretinogram, in the right eye and was nonrecordable from the left eye.

C O L O R

The single-flash full-field electroretinogram consisted of a reduced *b* wave with preserved *a* wave. The amplitude of the *a* wave was larger than that of the *b* wave in the right eye, making a negative-type electroretinogram. An electroretinogram could not be recorded from the left eye (Figure 2).

Discussion

Our patient was diagnosed with CSS because he had bronchial asthma, hypereosinophilia, radiographically detected migratory pulmonary opacities, and paranasal sinus abnormalities. These are four of the six diagnostic criteria for CSS proposed by the American College of Rheumatology in 1990.[1]

Acute blindness has been rarely described in cases of CSS, and a CRAO in cases of CSS has been reported in only a few cases (Table 1).[4,7–12] Our case showed not only retinal but also choroidal circulatory alterations. These findings suggest that the occlusion was probable in the ophthalmic artery. Udono et al[4] reported a similar case of CSS showing bilateral CRAO, although the CRAO developed in the left eye first and 6 days later in the right eye. Their case also demonstrated some choroidal circulatory compromise in both the eyes. To the best of our knowledge, the development of CRAO simultaneously in both the eyes has not been reported.

Several possible causes for the CRAO were considered. First, an increased thrombotic propensity because of eosinophilia may have caused the artery thrombosis.[12,13] Second, the inflammatory process of CSS vasculitis may involve the central retinal artery.[10] Finally, positive antiphospholipid serology hypercoagulable state, such as positive antiphospholipid serology[12] or high fibrin degradation products,[4] could be associated with the thrombotic process. In our case, no hypercoagulable state was observed, and neither retinal arterial emboli nor vasculitides were observed ophthalmoscopically or by fluorescein angiography as in most cases of CRAO reported (Table 1).[4,7,10] Because the underlying syndrome is usually manifested through vasculitis-induced organ damage, it cannot be denied that the retinal vascular occlusion might be associated directly or through attack on the posterior ciliary arteries with vasculitis. Bilateral ophthalmic arterial occlusion suggests that a relatively more central site is responsible in our case. However, it was not possible to determine a direct inflammatory infiltration or thrombotic event of the ophthalmic artery, and the exact pathophysiology is unknown.

The ocular manifestations in cases of CSS can be classified into two groups: the pseudotumor type (orbital inflammatory syndrome) and the ischemic vasculitis type.[2] The presence of ANCA is

Table 1. Previous Reports on Central Retinal Artery Occlusion Associated with Churg–Strauss Syndrome

Number	Author	Year	Reference Number	Age	Gender	Laterality	TIA	Retinal Findings: Embolus	Retinal Findings: Vasculitis	ANCA	Complication: Ocular	Complication: Systemic	Steroid	Visual Acuity: Initial	Visual Acuity: Final
1	Granata et al	2001	11	48	M	R	NM	NM	NM	+	Papilledema	—	80 mg/day	NM	No recovery
2	Udono et al	2003	4	68	M	R	NM.	—	NM	+	Choroidal circulatory compromise	High fibrin degradation products	HD	NLP	NLP
						L		—	NM		Choroidal circulatory compromise			NLP	NLP
3	Hoffman et al	2005	12	54	F	L	NM.	—	—	—	—	Moderate titer of anticardiolipin IgM	75 mg/day	LP	Improved peripheral vision
4	Hamann et al	2006	8	42	M	R	Yes	NM	NM	—	CRVO, NVG*	Hyperhomo-cysteinemia	50 mg/day	HM	No recovery
5	Türkçüoğlu et al	2007	7	44	F	L	Yes	—	NM	—	Cataract	—	HD	HM	HM
6	Skraparı et al	2008	10	50	F	L	NM	—	—	—	—	—	HD	LP	CF
7	De Salvo et al	2009	9	55	M	L	NM	NM	NM	NM	BRVO*, hypertensive retinopathy	—	75 mg/day	LP	LP

Visual acuity of the case reported by Granata et al is not clear. They reported just "vision loss."
*NVG was a late complication and BRVO was a preceded complication.
M, male; F, female; R, right; L, left; TIA, transient ischemic attack; NM, not mentioned; CRVO, central retinal vein occlusion; NVG, neovascular glaucoma, BRVO, branch retinal vein occlusion; :HD, high-dose therapy; NLP, no light perception; LP, light perception, HM, hand movement; CF, counting finger.

characteristic of the ischemic vasculitis type, which typically has a sudden onset of vision loss, a quiet-looking eye, and an absence of orbital imaging abnormalities. However, ANCA was negative in our patient whose clinical findings were more like those with the ischemic vasculitis type. In addition, there have been several cases of CSS that developed CRAO, but the ANCA score was negative (Table 1).[7,8,10,12] Retinal artery occlusion may not always occur as a characteristic manifestation in the ANCA-positive CSS patients. However, clinician should be aware that CRAO can develop in patients with CSS. Prophylactic steroid therapy should be considered even in ANCA-negative cases to prevent potential visual loss.

Key words: Churg–Strauss syndrome, ocular ischemia, central retinal artery occlusion, ocular artery occlusion.

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Incidence of Increased Intraocular Pressure after Subtenon Injection of Triamcinolone Acetonide

Ryosuke Kawamura,[1] Makoto Inoue,[1,2] Hajime Shinoda,[1] Kei Shinoda,[1,3] Yuji Itoh,[2] Susumu Ishida,[1,4] and Kazuo Tsubota[1]

Abstract

Purpose: To determine the incidence of eyes that have an increase in the intraocular pressure (IOP) after subtenon injections of triamcinolone acetonide (TA).
Methods: The medical records of 147 patients treated with single or multiple subtenon injections of TA (10 mg) were reviewed. The incidence of an IOP elevation (ΔIOP) ≥5 mmHg or an IOP of >21 mmHg was determined. The peak ΔIOP, defined as the difference in the IOP at the peak to the baseline IOP, was also evaluated. Multivariate analyses were used to evaluate the relation between the IOP elevation and the age, gender, refractive error, and lens status.
Results: A ΔIOP ≥5 mmHg was found in 75 eyes (46%), and an IOP >21 mmHg was found in 48 eyes (30%) after a single injection of TA. The IOP increased within 3 months in 39 eyes (81%) and after 4 months in 9 eyes (19%). The IOP began to increase significantly from 2 weeks up to 5 months ($P<0.05$) and returned to the baseline IOP in 10 months. The incidence of ΔIOP ≥5 mmHg or an IOP of >21 mmHg after multiple subtenon injections of TA was significantly higher than after a single injection (62%; $P=0.027$, 47%; $P=0.013$, respectively). The incidence of IOP >21 mmHg and the peak ΔIOP were significantly related with younger age ($P=0.002$, $P=0.021$, Forward stepwise regression analysis). A weak but significant negative correlation was found between the peak ΔIOP and the age ($r=-0.216$, $P=0.006$, Pearson's correlation coefficient test), and the peak ΔIOP and the refractive error ($r=-0.198$; $P=0.018$).
Conclusion: Repeated injections of TAs and injection of younger patients or myopic eyes increase the incidence of an IOP elevation.

Introduction

Corticosteroids are known to reduce inflammation, but systemic administration of corticosteroids can also lead to serious systemic side effects. For the eye, triamcinolone acetonide (TA), a corticosteroid, has been topically used to treat chalazions and eyelid angiomas, and the efficacy of intravitreal or subtenon injections of TA for various vitreoretinal diseases has been reported.[1–4]

An intravitreal injection is less frequently used because of the risk of endophthalmitis and retinal detachments, and a subtenon injection is preferred because of its safety and convenience.[5,6] It has been reported that an increase in the intraocular pressure (IOP) can develop after injections of TA and other corticosteroids to cause steroid-induced ocular hypertension.[7,8] Since TA is weakly water soluble and long-acting, the IOP elevations can occur several months after the injection, and the increase in the IOP can last for several months. This complication can result in some eyes having to undergo laser treatment and/or filtering surgery.[9–12] In spite of the earlier studies, the exact incidence of eyes that have an increase in the IOP after a subtenon injection of TA has not been published.[13]

The purpose of this study was to determine the incidence of an elevation of the IOP after single or multiple injections of TA. To accomplish this, we reviewed the medical records of cases that had received either single or multiple subtenon injections of TA for several retinal disorders associated with major retinal vascular pathologies.

Methods

Subjects

A retrospective case series study was conducted on 162 eyes of 147 patients (79 men, 68 women) who had received single or multiple subtenon injections of 10 mg TA. There

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were 38 eyes of 26 patients with diffuse diabetic macular edema, 64 eyes of 63 patients with branch retinal vein occlusion, 10 eyes of 9 patients with central retinal vein occlusion, 39 eyes of 38 patients with exudative age-related macular degeneration, 7 eyes of 7 patients with idiopathic choroidal neovascularization, and 4 eyes of 4 patients with macular edema by other ocular diseases that were treated with TA.

The patients had been treated at the Keio University Hospital between June 2002 and January 2007 and were followed for at least 4 months. None of the patients had received an earlier subtenon injection of other corticosteroids. The baseline IOP was measured at least twice at different times on different days before the injection, and patients in whom glaucoma was diagnosed or had a baseline IOP >21 mmHg were excluded. The anterior chamber angle of all patients was open without any evidence of neovascularization.

A written informed consent was obtained from all patients after a full explanation of the purpose and possible complications of the subtenon TA injection. The Ethics Committee of Keio University approved the use of TA including possible additional treatments based on recommendation of the Institutional Review Board. The procedures used conformed to the tenets of the Declaration of Helsinki.

Procedures for subtenon TA injections

To prepare 10 mg of TA, 0.25 mL containing 10 mg of TA (Kenacort-A®, 40 mg/mL; Bristol Myers KK, Tokyo, Japan) was aspirated into a 1 mL syringe. The syringe was placed in a holder for several minutes, and after the crystals of TA had settled on the bottom, the supernatant was reduced to a volume of 0.1 mL. Immediately before the subtenon injection, the mixture in the syringe was shaken well to make a uniform suspension of the TA crystals.

Before the TA injection, the eye was anesthetized with several drops of 4% lidocaine. An eye speculum was used, and the patient was asked to look inferonasally to expose the superotemporal bulbar conjunctiva. A 26-gauge needle was inserted superotemporally into the subtenon space by gently moving the tip of the needle to avoid penetrating the globe. The tip of the needle was not sharp and was similar to the needle attached to a tuberculin syringe.

IOP measurements

The IOP was measured by Goldmann aplanation tonometry. Patients were examined at ~2 weeks and at 1 month after the TA injection. Thereafter, the follow-up examinations were done monthly. The incidence of IOP elevation (ΔIOP) ≥5 mmHg from the IOP before the injection (baseline IOP) and the incidence of IOP increased to >21 mmHg in any period after TA injection were evaluated. The peak ΔIOP, defined as the difference in the IOP at the peak elevation from the baseline IOP, was also evaluated. If the IOP exceeded 25 mmHg, topical beta-blockers, prostaglandin derivatives, or carbonic anhydrase inhibitors were given. If the IOP continued to be elevated, different combinations of these drugs were used. Selective laser trabeculoplasty (SLT) was usually performed if the IOP continued to be higher than 40 mmHg despite the maximum medication including systemic carbonic anhydrase inhibitors. The eyes that underwent SLT were included in the analysis on the ΔIOP but not included in the analysis evaluating the course of the IOP elevation after the treatments with SLT. The eyes that underwent cataract and/or vitreous surgery during the observation period or simultaneously with the TA injection were excluded to eliminate bias of the host factors.

The topical medication score was defined as the number of anti-glaucoma eye drops being used, and the scores were assessed before and after the TA injection. Statistical analyses were performed by a commercial statistical software package. Paired *t*-tests were used to assess the significance of differences in the IOPs before and after the TA injections. Fisher's exact probability tests were used to evaluate the significance of the ΔIOP. Forward stepwise regression analysis was used to evaluate the relationship between IOP elevation (IOP >21 mmHg or ΔIOP ≥5 mmHg, or the peak ΔIOP) and age, gender, refractive error, and lens status. Pearson's correlation coefficient test was used to determine the relationship between the age and the refractive error, or the age and the peak ΔIOP. The level of significance was 0.05 for all statistical tests.

Results

Incidence of elevation in IOP

A total of 311 subtenon TA injections were given to the 162 eyes. The mean age of the patients was 62.9±11.9 years (±standard deviation) with a range from 30 to 86 years. The mean baseline IOP before a single TA injection was 14.4±3.5 mmHg with a range from 8 to 21 mmHg (Table 1). A ΔIOP ≥5 mmHg was found in 75 eyes (46%), and an IOP >21 mmHg was found in 48 eyes (30%) after a single injection of TA. After a single injection of TA, a ΔIOP ≥5 mmHg was observed at 2 weeks to 8 months (mean 2.2 months) after the injection (Fig. 1). The mean IOP was elevated significantly for 2 weeks to 5 months. The mean peak ΔIOP was 17.3±6.0 mmHg (range from 8 to 62 mmHg), which was attained at 2 months after the TA injection. The mean IOP

TABLE 1. INCIDENCE OF INTRAOCULAR PRESSURE ELEVATION AFTER SUBTENON TRIAMCINOLONE ACETONIDE INJECTION

	Numbers of eyes	*baseline IOP (mmHg)*	*ΔIOP≥5 mmHg*	*IOP>21 mmHg*	*IOP>30 mmHg*
Total injections	162	14.4±3.5	101 (62%)	73 (45%)	17 (10%)
Initial single injection	162	14.2±2.9	75 (46%)[a]	48 (30%)[b]	13 (8%)[c]
Repeated multiple injections	77	14.6±3.5	48 (62%)[a]	36 (47%)[b]	5 (6%)[c]

[a]$P=0.027$.
[b]$P=0.013$.
[c]$P=0.797$, Fisher's exact probability test.
IOP, intraocular pressure; ΔIOP, IOP elevation from the baseline IOP.

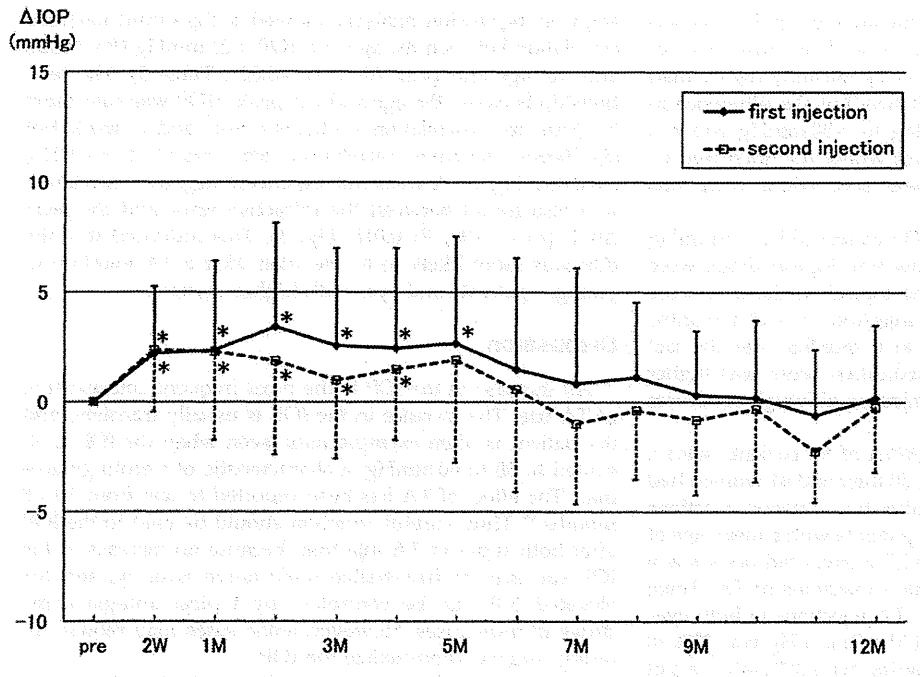

FIG. 1. Mean intraocular pressure (IOP) before and after a single and a second injection of TA. The IOPs at 2 weeks, and 1, 2, 3, 4, and 5 months after the injection of TA are significantly higher than the baseline IOP after a single injection. The IOPs at 2 weeks, and 1, 2, 3, and 4 months are significantly higher than the baseline after a second injection (*$P<0.05$, error bar: standard deviation, Paired t-tests). W, week, M, month; TA, triamcinolone acetonide.

decreased to 15.8±4.7 mmHg (ranged 9 to 35 mmHg) at 6 months after the TA injection, and it was not significantly different from that of the baseline IOP. The mean IOP returned to 14.2±3.1 mmHg (approximate the mean baseline IOP) with reduced numbers of medications at 10 months after the TA injection.

The IOP began to increase within 3 months in 60 (80%) of the 75 eyes with an IOP elevation ≥5 mmHg after the single TA injection. The increase in the IOP began at 4 months after the injection in the other 15 eyes (20%). The IOP increased to >21 mmHg after a single injection within 3 months in 39 eyes (81%) and in 4 months in the remaining 9 eyes. In 13 of the 162 eyes (8%), the IOP increased to >30 mmHg at 4 months after the injection.

In the 75 eyes with a ΔIOP ≥5 mmHg after a single injection, topical anti-glaucoma medications were given to 33 eyes (44%). The topical medication score was 0 at baseline and increased to 1.1 at 5 months after the single injection (Fig. 2). The score gradually decreased to 0.2 at 10 months after the injection. After a single subtenon injection of TA, SLT was performed on 3 eyes, and the IOPs of all 3 eyes were controlled without any medication at 6 months after the TA injection.

In the eyes that required 2 TA injections, the IOP increased significantly from 2 weeks after the second injection, but the IOP was not significantly higher at 5 months after the injection (Fig. 1). Seventy-seven of the 162 eyes received multiple subtenon injections of TA (Table 1). The incidence of a ΔIOP ≥5 mmHg after multiple subtenon injections of TA

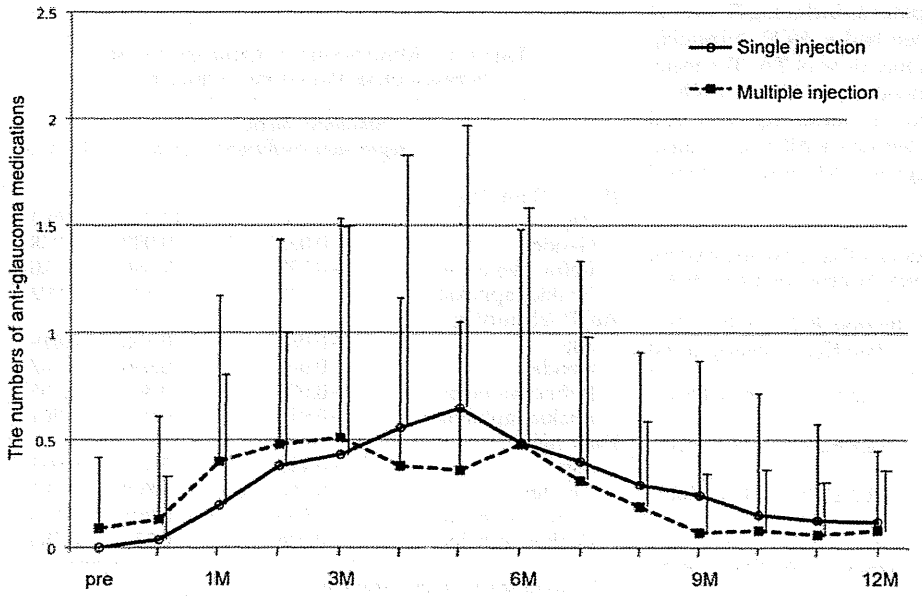

FIG. 2. Topical medication score before and after single and multiple injection of TA. The mean number of glaucoma medications increases from 0 to 1.1 at 5 months after the single injection and gradually decreased to 0.2 at 10 months after the injection. The topical medication score was <0.1 before the multiple injections, 0.5 at 4 months after the injection, and 0.2 at 8 months after the injection. The topical medication score rapidly increased compared with eyes with the single injection (error bar: standard deviation, Paired t-tests).

was significantly higher than that after a single injection ($P=0.027$, Fisher's exact probability test). The incidence of an IOP increasing to >21 mmHg was significantly higher than that after a single injection ($P=0.013$), but the difference in the incidence of an IOP increasing to >30 mmHg was not significant ($P=0.797$). In the 5 eyes whose IOP increased to >30 mmHg, the IOP elevation was seen within 3 months after the last injection.

Among the 48 eyes whose ΔIOP increased by ≥5 mmHg after multiple injections, anti-glaucoma topical drops were prescribed to 22 eyes (46%). The topical medication score was <0.1 before the additional injections, 0.5 at 4 months after the last injection, and 0.2 at 8 months after the last injection (Fig. 2). The topical medication score was higher than that after the initial single injection although the scores were relatively low.

One hundred and one eyes (62%) of 91 patients with a mean age of 63.3 years including 50 men and 41 women had a ΔIOP ≥5 mmHg at least once after the subtenon injections of TA. Sixty-one eyes (38%) of 56 patients with a mean age of 62.9 years including 29 men and 27 women did not show a ΔIOP ≥5 mmHg after the subtenon injections of TA. There were 15 cases that had subtenon TA injections in both eyes (Table 2), and the incidence of IOP >21 mmHg was 27% (8 eyes) of bilateral injections comparing with 30% (40 eyes) of unilateral injections ($P=0.826$, Fisher's exact probability test). In 8 eyes with bilateral injections with IOP increased to >21 mmHg, the IOP increased bilaterally in 3 cases (60%) and unilaterally in 2 cases (40%). However, there was a selection bias because both eyes from 15 patients were included in this analysis.

Relationship between change in IOP elevation and refractive error

One hundred and forty-two of the eyes (88%) of 127 patients were phakic, and 20 eyes of 20 patients were pseudophakic. None of the eyes was aphakic. The effect of the refractive error was only evaluated for the phakic eyes. The mean refractive error in the phakic subjects was −2.3±3.8 diopters (D) with a range from −19.0 D to +2.5 D. In 142 phakic eyes, 91 eyes (64%) of 85 patients including 55 eyes of 50 men and 36 eyes of 35 women had a ΔIOP ≥5 mmHg after single or multiple subtenon injections of TA. The mean refractive error (spherical equivalent) with a ΔIOP ≥5 mmHg was −2.4±3.3 D, and the mean age was 60.5 years. The mean refractive error without a ΔIOP ≥5 mmHg was −2.1±4.7 D, and the mean age was 62.3 years. Forward stepwise regression analysis showed a significant negative correlation between the age and IOP >21 mmHg ($P=0.002$), and the age and peak ΔIOP ($P=0.021$, Table 3). The relationship between the age and the peak ΔIOP was calculated by Pearson's correlation coefficient test, and a weak but significant negative correlation was found ($r=-0.216$, $P=0.006$; Fig. 3). A weak but significant negative correlation was also found between the refractive error and the peak ΔIOP ($r=-0.198$, $P=0.018$; Fig. 4). This indicated that the IOP was more likely to be elevated after a TA injection in younger patients and eyes with higher myopia.

Table 2. Incidence of Intraocular Pressure Elevation after Subtenon Triamcinolone Acetonide Injection

	Number of cases	*Number of eyes*	*Baseline IOP (mmHg)*	*IOP >21 mmHg (eyes)*
Unilateral Injection	132	132	14.2±3.0	40 (30%)[a]
Bilateral injections	15	30	14.6±2.7	8 (27%)[a]
Total injections	147	162	14.2±2.9	48 (30%)

[a]6 eyes of 3 cases and 2 eyes of 2 cases, $P=0.826$, Fisher's exact probability test.

Discussion

An increase in the IOP is the most frequent complication of TA use. The increase in the IOP is usually transient, and the patient is often asymptomatic even when the IOP is elevated to 50 to 60 mmHg, a characteristic of steroid glaucoma.[7] The effect of TA has been reported to last from 3 to 8 months.[14] Thus, careful attention should be paid to the IOP after both types of TA injection, because an increase in the IOP can lead to irreversible optic nerve atrophy, and the elevated IOP can be controlled by topical anti-glaucoma drugs in most cases. However, some cases may require filtering surgery to normalize the IOP.

Helm and Holland reviewed 20 eyes that had received subtenon injections of TA, and they reported that an elevated IOP was found in 6 patients (30%), and 2 of the 6 eyes had received several injections.[13] Mueller and associates studied 54 steroid nonresponders and reported that a subtenon injection of TA (40 mg) was safe and did not cause an increase in the IOP when the deposit was injected into the subtenon space.[15] Levin and associates reported an increase in the IOP in 4 of 9 patients (44%) who were steroid-responders and 7 of 55 patients (13%) who were steroid nonresponders.[16] Some patients had an IOP elevation after a subtenon injection of TA even in steroid nonresponders. These findings indicated that the conventional steroid response test cannot clearly differentiate the eyes that will have an elevation of the IOP after subtenon TA.

Table 3. Multivariate Analysis with Intraocular Pressure Increase

	Standard partial regression coefficient	F *value*	P *value*
IOP >21 mmHg			
Age	−0.266	10.313	0.002
Gender	−0.023	0.087	0.768
Refractive error	−0.122	2.446	0.120
Phakia/aphakia	0.140	2.872	0.092
ΔIOP ≥5 mmHg			
Age	−0.069	0.656	0.419
Gender	0.067	0.690	0.407
Refractive error	−0.075	0.861	0.355
Phakia/aphakia	−0.015	0.031	0.861
Peak ΔIOP			
Age	−0.196	5.473	0.021
Gender	0.106	1.808	0.181
Refractive error	−0.035	0.201	0.655
Phakia/aphakia	0.028	0.114	0.736

Forward stepwise regression analysis.

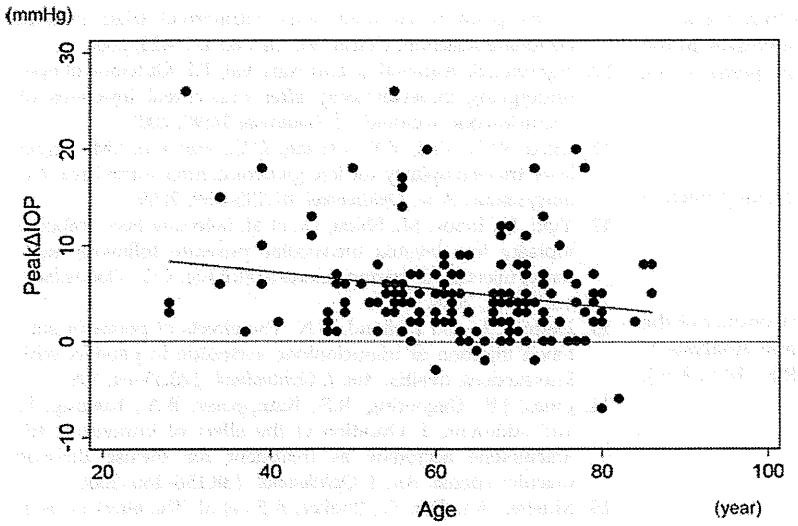

FIG. 3. Relationship between the change in the peak ΔIOP (the peak elevation from the baseline IOP) and age. A significant negative correlation exists between the peak ΔIOP and the age, indicating that the IOP increased in younger patients more often than in older patients ($P=0.006$; $r=-0.216$, Pearson's correlation coefficient test). ΔIOP, incidence of IOP elevation.

Ito and associates reported that the IOP increased to >21 mmHg in 41 eyes (34.5%) of 119 eyes after intravitreal and subtenon injections of TA, and an IOP increased by >5 mmHg from the baseline was found in 59 eyes (49.6%).[17] It has also been reported that the ΔIOP was higher in younger patients and also after repeated injections.[17] Jonas and associates also reported that the IOP increased to >21 mmHg in 39 (52%) of 75 eyes after an intravitreal injection of TA, and one of these cases required trabeculectomy.[7,18] The dose of TA for subtenon injection was usually 20 to 40 mg, which was more than that of an intavitreal injection of 4 to 20 mg. An intravitreal injection of TA is believed to affect the trabecular meshwork in the angle more directly than the subtenon injection. This may be why a delayed IOP elevation was observed after subtenon TA injection in our study.

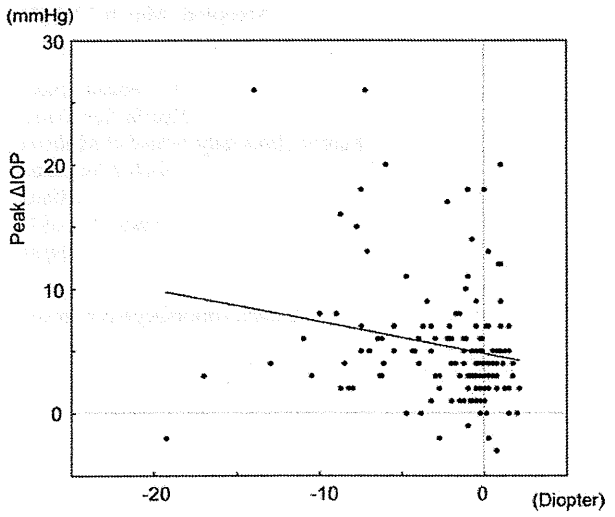

FIG. 4. Relationship between the change in the peak ΔIOP (the peak elevation from the baseline IOP) and refractive error. A significant negative correlation exists between the peak ΔIOP and the refractive error, indicating that the IOP in myopic eyes increased more often than in eyes with higher refractive errors ($P=0.018$; $r=-0.198$, Pearson's correlation coefficient test).

We injected the TA into the posterior subtenon space around the equator of the eye, and 46% of the cases had an elevated IOP within 2 months after the initial subtenon injection of TA. By 3 months, 80% of the eyes had developed an IOP elevation. However, cases of late-onset IOP elevation were present in our series, and repeated injections of TA may increase the risk of an IOP elevation. An IOP elevation was observed in 20% of our cases beginning 4 months after the TA injection. This suggests that a repeat TA injection within 4 months of a previous injection might increase the risk of IOP elevation. There may be a potential bias in our delay in giving the second injection when the IOP had risen after the initial injection. Thus, the incidence of IOP elevation after repeated injections would be higher than that stated earlier. Thus, repeated injections should be considered more carefully to avoid the risk of ocular hypertension.

A significant correlation was found between an IOP elevation and younger age and gender.[19] A weak but significantly negative correlation between refractive error and IOP elevation was found in our cases, indicating that more attention should be paid to younger patients and those with high myopia. In an earlier report, the authors recommended that patients should be tested for the presence of a positive steroid response by applying a topical steroid before the use of TA, and TA should be avoided if the patient was a steroid-responder.[16] However, our results showed that the IOP elevation could be controlled in most of the cases by topical anti-glaucoma drugs. In addition, we found that some patients had an elevation of the IOP unilaterally after a bilateral injection of TA. Thus, we believed that an elevated IOP after TA is probably more complex than expected from the presence of topical steroid response. The mechanism for the significant correlation between high myopia and an elevation of the IOP requires further study.

In conclusion, a subtenon injection of TA has been used for treatment, but can be a high risk of an IOP elevation. However, the elevated IOP is transient. We recommend frequent measurements of the IOP and long-term observations

in patients who have had a subtenon or an intravitreal injection of TA, because cases with late-onset increases in the IOP can be present and retreatment with TA increases the risk of IOP elevation.

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Author contributions

Design of the study (R.K., M.I., K.S., K.T.); Conduct of the study (R.K., M.I., K.S., S.I., K.T.); Collection and Analysis of the data (R.K., H.S., Y.I.); Literature Search (R.K., M.I., K.S.).

Author Disclosure Statement

No competing financial interests exist.

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Stiles–Crawford effect in focal macular ERGs from macaque monkey

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Background: To determine whether the focal macular electroretinograms (FMERGs) are affected by the angle of incidence of the stimulating light on the retina, i.e., the Stiles–Crawford effect (SCE). *Methods*: FMERGs were elicited by focal stimulation of the macula in three light-adapted macaque monkeys. The incidence of the light on the retina was varied from 0 to ±11.7°. The effects of the incidence and wavelengths of the stimulus on the SCE were determined. *Results*: The amplitudes of the FMERG components were largest when the stimulus beam entered the eye on the visual axis and passed through the center of the pupil. The amplitudes gradually decreased as the stimulus beam passed through the pupil more eccentrically and fell on the retina more obliquely. All components of the FMERGs were decreased with the decrease least for the amplitude of the d-wave. *Conclusions*: The decrease in the amplitudes of the FMERGs as the angle of incidence of the stimulus beam on the retina increases demonstrates that the SCE can be detected in adult macaque monkeys. This objective method of assessing the SCE suggests that this technique can be used to assess the alignment of cones in humans with different types of macular diseases.

Keywords: Stiles–Crawford effect, focal macular electroretinogram, ERG

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Introduction

Focal macular electroretinograms (FMERGs) have been used to assess the physiological condition of different retinal neuronal cells including the photoreceptors in the macular area (DeLint, Berendschot, & van Norren, 1998; Kondo, Miyake, Horiguchi, Suzuki, and Tanikawa, 1998). In most experimental and clinical studies, FMERGs have been recorded, and the effects of the Stiles–Crawford effect (SCE), a decrease in the luminous efficiency of light entering the edge of the pupil, were not examined. However with focal stimulation, the direction of the incidence beam becomes more important.

Evidence has been obtained that the directional sensitivity of the cones to light stimuli is responsible for the SCE (Alpern, 1986; Alpern, Kitahara, & Fielder 1987; Alpern & Kitahara, 1983; Stiles & Crawford, 1933). The SCE is generally determined by psychophysical tests (Alpern, 1986; Alpern et al., 1987; Alpern & Kitahara, 1983; DeLint, Vos, Berendoschot, & van Norren, 1997), and the subjects are required to actively participate in the examination. Thus, high level of concentration and good visual acuity are required to perform the tests.

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The SCE has been examined objectively in humans by only a few investigators. DeLint et al. analyzed the SCE by measuring the visual pigment density with different incidences of the bleaching and reflected light (DeLint et al., 1998). Birch et al. stimulated focal macular areas with a steady-state flickering light through a two-channel Maxwellian-view optical system to elicit focal ERGs (Birch, Sandberg, & Berson, 1982). They showed that the SCE could be demonstrated at the fovea in normal subjects. However, they isolated the b-waves by using band-pass filters, and a separation of a-wave and d-wave components was not possible. The FMERGs in our study enabled us to isolate the a-, b-, and d-waves, and we were able to analyze each component to determine whether each showed the SCE effect.

We have developed an FMERG system that is integrated into a slit lamp that allowed us to stimulate the retina with a spot of light at any incidence (Choshi, Matsumoto, & Nakatsuka, 2003; Yamada, Matsumoto, & Nakatsuka, 2006). This FMERG stimulating and recording system prompted us to assess the SCE objectively with long-duration stimuli. This system allowed the ERG recordings to be performed under direct fundus observation, and each component of the FMERGs could be analyzed separately.

Thus, the purpose of this study was to determine whether the SCE was present in the foveal area of macaque monkeys. To accomplish this, we elicited FMERGs from three macaque monkeys by light of different incidences on the retina. We shall show that all components of the FMERGs were affected by the SCE with the d-wave least affected.

Materials and methods

Animals

Focal macular ERGs (FMERGs) were recorded from 3 eyes of 3 adult (ages 6, 6, and 8 years) male macaque monkeys (*Macaca fuscata*). All experimental and animal care procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Institutional Animal Care Committee of Oita University.

FMERG recordings

The monkeys were initially anesthetized by an intramuscular injection of a mixture of ketamine (7 mg/kg), xylazine (0.6 mg/kg), and butorphanol tartrate (0.04 mg/kg) and maintained on an infusion of the same proportions of ketamine, xylazine, and butorphanol tartrate per hour. The depth of the anesthesia was maintained at a level sufficient to inhibit the corneal reflex and eye movements. The pupils were dilated with topical tropicamide (0.5%) and phenylephrine hydrochloride (5%), and the cornea was anesthetized with topical oxybuprocaine hydrochloride (0.4%). The non-stimulated eye was covered with an opaque patch.

Photopic stimuli

An optical system was built to deliver focal stimuli to the macula under direct fundus observation. The system was designed so that the stimulus light passed through the center of the pupil, i.e., on the visual axis, or through different parts of the pupil from the temporal to nasal edge in 0.5-mm steps. The light sources were from hyper-bright light-emitting diodes (LEDs; NSPW500BS, NICHIA, Tokushima, Japan), and the stimuli positioning unit was a motorized helicoid stage (Sigma Koki, Saitama, Japan) with a telescopic optical system mounted on the stage. The stage moved the telescope so that the stimulating beam entered the pupil from the temporal to nasal sides across the visual axis in 0.5-mm steps. The movement of the stage had an accuracy of 0.05 mm.

The fundus observation system was composed of a near-infrared CCD camera (Hitachi, Japan) integrated on a customized slit lamp microscope (Carl-Zeiss, Germany). The position of the light spot on the macular area was monitored during all of the recordings. The stimulus spot was 5° in diameter. To examine the effects of the wavelength of the stimulus on the SCE, red (λ_{max} = 644 nm, half-amplitude bandwidth of 634 to 655 nm, TLRH180P, TOSHIBA, Tokyo, Japan), amber (λ_{max} = 590 nm, half-amplitude bandwidth of 585 to 596 nm, TLYE260A, TOSHIBA, Tokyo, Japan), green (λ_{max} = 523 nm, half-amplitude bandwidth of 512 to 545 nm, SLA580EC4T, ROHM, Kyoto, Japan), and blue (λ_{max} = 470 nm, half-amplitude bandwidth of 460 to 482 nm, NSPB500S, NICHIA, Tokushima, Japan) LEDs were used to elicit the FMERGs.

The white light stimulus intensity was set to 38 cd/m^2. The spectral characteristics (bandwidth and λ_{max}) of the LEDs used in this study were measured with a spectral colorimeter PR-650 SpectraScan and analyzed with SpectraView software (Photoresearch, CA, USA).

To determine whether the ERGs were focal, the 5° stimulus spot was projected onto the optic nerve head, and FMERGs were elicited by decreasing stimulus intensities. The FMERGs recorded by the stimulus projected on the optic nerve head became non-recordable when the intensity was ≤38 cd/m^2 indicating that this stimulus intensity would provide a focal response from the macula with negligible effect of stray light (Choshi et al., 2003; Yamada et al., 2006). The intensity of each colored light stimulus was matched by neutral density filters to elicit approximately the criteria amplitude of b-wave (1 μV) as elicited by the white stimulus whose luminance was

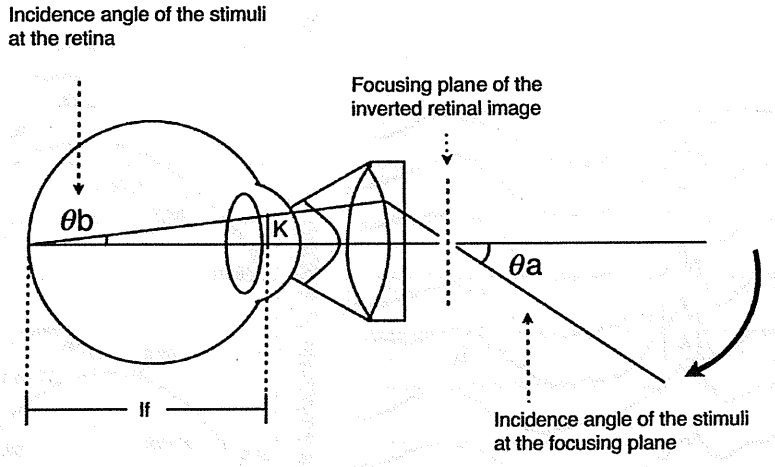

Figure 1. Relationship between the angle of incidence of the light beam on the focusing plane of the inverted retinal image. IF = IF distance, i.e., the axial length minus the corneal thickness and anterior chamber depth and K = distance of the stimulus beam from the visual axis in the iris plane in millimeters.

$\leq$38 cd/m^2. The FMERG waveforms were comprised of on and off waves. The adjusted intensity elicited no ERGs when the stimulus spot was projected onto the optic nerve head.

A gold-foil bipolar contact lens (Mayo, Nagoya, Japan) coupled with a mini-pan fundus lens was placed on the cornea of the examined eye (Figure 1). This provided an inverted real image of the ocular fundus projected approximately 3.5 mm in front of the contact lens unit. The relationship between the angle of incidence of the light beam on the focusing plane of the inverted retinal image and that on the retinal surface was calculated as shown in Figure 1. When the light beam fell on the temporal side of the real image at an angle of θ_a, then the light beam will be projected from the nasal side onto the retinal surface at an angle of θ_b. The relationship between θ_a and θ_b was calculated by

$$[\tan\theta_b = C(\tan\theta_a)], \quad (1)$$

with θ_b being the angle of incidence on the retina, θ_a being the angle of incidence on the focusing plane of the inverted retinal image, and C being the lens magnification constant. Thus, θ_b was determined by the telescope angle and C by the lens magnification constant (0.39).

The distance between the center of the pupil and the stimulus beam was measured as shown in Figure 1. The iris–fovea distance, IF, was calculated by

$$\mathrm{IF} = K/(\tan\theta_b), \quad (2)$$

with K equal to the distance of the light beam from the visual axis in the iris plane, and IF equal to the distance of the axial length with the subtraction of corneal thickness and the anterior chamber depth. A correction for light transmission through the cornea and the lens was not done. The axial length, corneal thickness, and the anterior chamber depth were measured by A-mode ultrasound echography (Compuscan LT, Stortz, St. Louis, MO, USA).

The stimulus duration was 100 ms and the stimulus interval was 150 ms, and thus, the frequency of stimulation was 4 Hz. This stimulus pattern of 100 ms on and 150 ms off was used for each wavelength stimulus. A white light of 35 cd/m^2 for 15 min was used for light adaptation before recording with each wavelength. We believe that the off duration was long enough because the depolarization of the cone is much faster than 150 ms.

FMERG recordings and analyses

A bipolar contact lens electrode (Mayo, Nagoya, Japan) was used to pick up the FMERGs. The ground electrode was attached to the right ear lobe. After centering and focusing the stimulus spot on the macula, the eye was light adapted with 35 cd/m^2 for 15 min. Then, the FMERGs were elicited by different stimulus intensities and wavelengths. The responses were amplified with a NeuropackΣ bioamplifier (MEB-5500, Nihon Kohden, Tokyo), A/D converted at 16 bits (PCI-16/16UD, Contec, Japan), and averaged by a customized signal processing program (MTS, Japan). More than forty responses were averaged, and the sampling rate was 10 kHz (every 0.1 ms). The responses were filtered from 0.5 to 200 Hz with a hardwired band-pass filter. With this system, the noise level with the electrode placed on the cornea and no stimulus was less than 0.1 μV.

The amplitude of the a-wave was measured from the baseline to the trough of the a-wave, and the amplitude of the b-wave was measured from the trough of the a-wave

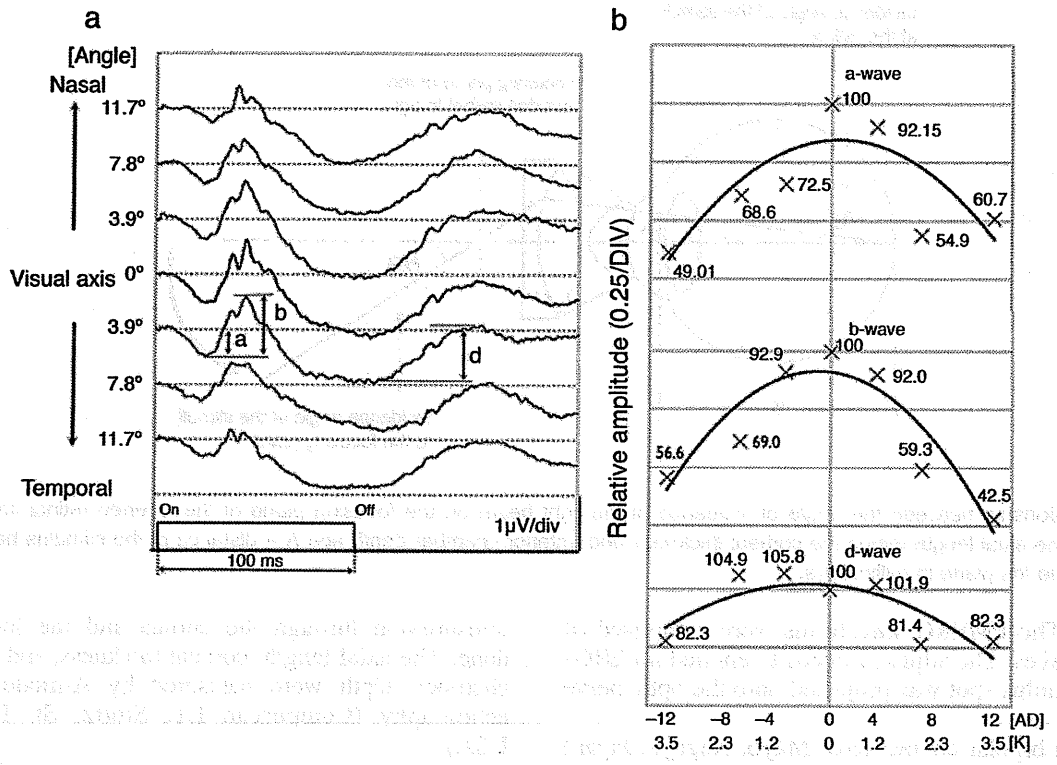

Figure 2. (a) Representative FMERGs elicited by white stimuli entering the pupil at different distances (ordinate) from the visual axis (0) for up to 11.7 degrees. The amplitudes of the a- and b-waves of the FMERGs decrease with increasing distance from the visual axis. (b) The amplitudes of the a-, b-, and d-waves were measured from ERGs such as those from the three monkeys, and the relative amplitudes are plotted on the right. One division of the graph is equal to 25%. *K* represents the distance of the entrance beam to the visual axis in the iris plane in millimeters; AD represents the mean angle of incidence in degrees of stimulus spot from the axis. The blue LED has a peak at 470 nm with a half-amplitude bandwidth between 442 and 520 nm. The green LED has a peak at 523 nm with a half-amplitude bandwidth between 480 and 620 nm. The yellow–orange LED has a peak at 590 nm with a half-amplitude bandwidth between 470 and 620 nm. The red LED has a peak at 644 nm with a half-amplitude bandwidth between 580 and 675 nm.

to the following positive peak (Figure 2). The amplitude of the d-wave was measured from the trough just preceding the d-wave to the positive peak after the stimulus offset.

All of the results are expressed as means ± standard deviations (*SD*s). The polynomial fit of the data was made with the Excel program, ver. 12.0. The polynomial fit was at order 2 for all data.

Results

The amplitudes of the a- and b-waves of the FMERGs were largest when the stimulus beam entered the eye on the visual axis ($K = 0$ mm, retinal angular incidence = 0 degree), and they decreased progressively as the stimulus beam entered more eccentrically up to the edge of the pupil. For example with white light, the average ($n = 3$) relative amplitude of the a-wave at 11.7° was approximately one-half of that at 0°. In the same way, the average ($n = 3$) of the relative amplitude of the b-wave at 11.7° was approximately one-half of that at 0°. Similar changes were found for the d-wave although the degree of change (reduced by 17.7 to 18.6%) was smaller (Figure 2 and Supplementary Table 1). With other wavelengths, the changes depended on the stimulus angle, but the relative amplitude of the d-wave was not evident.

The relative amplitudes of the FMERGs elicited by different wavelengths with peak transmission at 470 nm, 524 nm, 590 nm, and 644 nm are shown in Figure 3. As with the white stimuli, the amplitudes of the a- and b-waves were largest when the light beam entered the pupil on the visual axis and decreased with greater eccentricities. However, the degree of the SCE was not significantly affected by the wavelength of the stimuli (Figure 3).

When the relative amplitude difference was compared between the b-wave and d-wave, significant differences were found for each wavelength used. The relative amplitude differences was calculated as the relative amplitude by the stimulus at 0*K*—that by the stimulus at

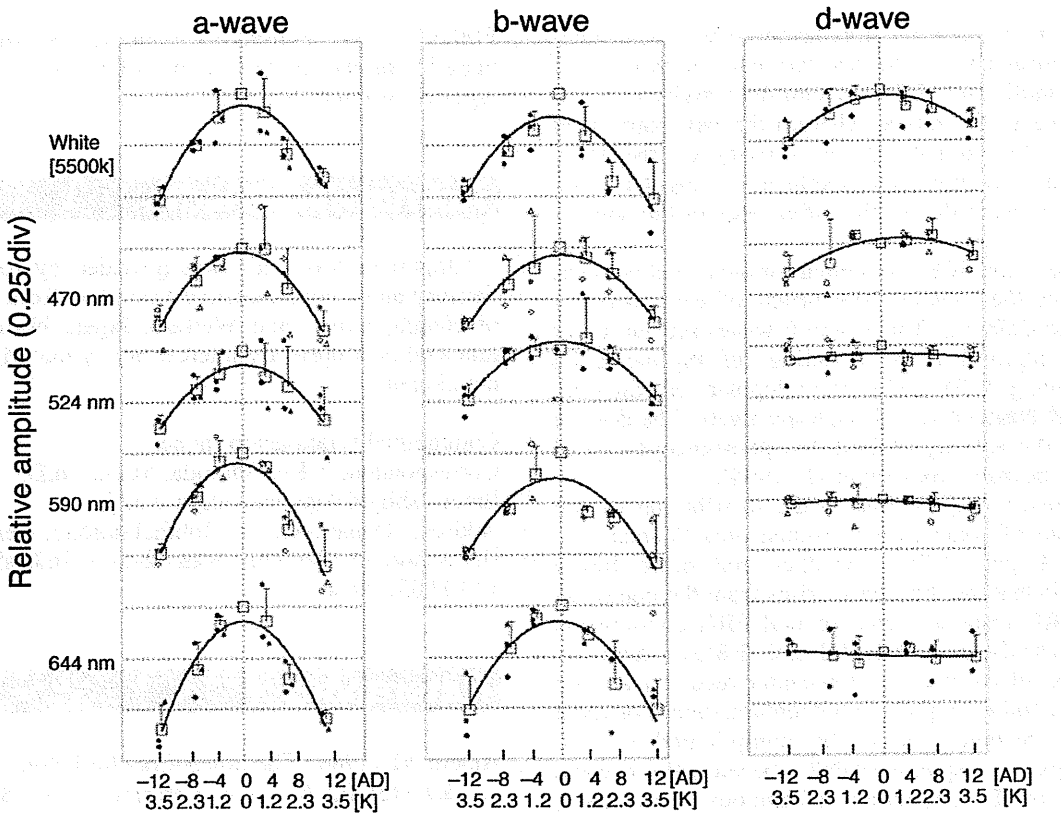

Figure 3. Relative amplitudes of the a-, b-, and d-waves elicited by different wavelengths of the stimulus. The λ_{max} of the stimuli was at 470 nm, 524 nm, 590 nm, and 644 nm. The small symbols (circles, triangles, and asterisks) represent the individual monkeys, the large squares are the averages, and the bars are the standard error of the means. One division of the graph is equal to 25%. *K represents the mean distance of the entrance beam to the visual axis in the iris plane in millimeters; †AD represents the mean angle of incidence in degrees of stimulus spot from the axis.

3.5K (Supplementary Table 1). The relative amplitude difference between maximal incidence stimuli and minimal incidence stimuli was significantly larger for the b-wave than for the d-wave for all wavelengths (Supplementary Table 1). Thus, the d-wave amplitude at the maximal stimulus incidence did not decrease as much as that of the b-wave suggesting that the SCE was larger for the b-waves than for the d-waves.

Discussion

Our results showed that the amplitudes of the a-wave and b-waves of the FMERGs were largest when the stimulus beam entered the eye through the center of the pupil and struck the retina perpendicularly. The amplitudes decreased progressively with increasing eccentricities of the stimulus beam. This is comparable to the SCE obtained psychophysically, and our results demonstrated that the SCE can be measured objectively using the FMERGs to assess the response of the retina.

The SCE is based on the ability of the photoreceptors to absorb photons passing through the outer segments, and the chances of a photon striking a photopigment increase when the beam passes perpendicularly through the entire extent of the outer segments. This explains why the FMERGs are largest when the stimulating beam entered the eye along the visual axis.

More than 25 years earlier, Birch et al. (1982) showed that the SCE can be demonstrated in the focal ERGs elicited by flickering stimuli obtained from a two-channel Maxwellian-view optical system. Our results confirmed their findings and also provided new information on the characteristics of the different components of the ERG and the influence of the wavelength of stimulating light.

Our data showed a drop-off in amplitude of the FMERGs when elicited by stimuli entering the edge of the pupil was approximately 50% whereas it was reported that the decrease of sensitivity was 1 log unit psychophysically (Alpern & Kitahara, 1983) or by OCT (Gao, Cense, Zhang, Jonnal, & Donald, 2008). The discrepancy between perimetry or OCT and the FMERGs is most likely due to methodological differences, i.e., the

FMERGs were elicited by suprathreshold stimulus intensities from focal areas, whereas perimetry employs near threshold stimuli. The FMERGs sum the activity of all of activated cells in the retina, whereas the psychophysical threshold is determined by the activity of the most sensitive cells. An alternative explanation might be that the differences were due to the differences in the species studied.

The degree of the SCE was similar for the a- and b-waves but lower for the d-wave. The origin of each wave is thought to be different. The a- and b-waves receive input from postreceptoral cells including off bipolar cells (Bush & Sieving, 1994) and from on bipolar cell (Sieving, Murayama, & Naarendorp, 1994), respectively. The d-wave arises from the activity of both the photoreceptors and inner retinal neurons (Sieving et al., 1994).

The difference may be due to the distribution of the different types of cones in the macular area (Yamamoto, Gouras, & Lopez, 1995). Another and more likely explanation is that the difference arises from the complexity of the ERG responses with several ERG components interacting. The d-waves of the full-field ERGs result from an interaction of the cone photoreceptor recovery and on bipolar offset and off bipolar cell depolarization. For focal stimuli, the response might be complicated by the photopic negative response (PhNR), because the d-wave is abolished by TTX treatment (Kurimoto et al., 2009; Yamada et al., 2006). Further investigations must be done to determine the reason for the different properties of the d-waves.

Stiles (1937) showed that the SCE depended on the wavelength of the light stimuli, and Alpern and Kitahara (1983) demonstrated that the directional sensitivity parameter (P) is dependent on the wavelength, namely, P was high at short (400 nm) and long (700 nm) wavelengths and lower at middle (550 nm) wavelengths. Our methods were not sensitive enough to detect these differences for the different wavelengths of the stimuli, probably because the bandwidths of the stimuli were relatively broad in our study. This was a limitation of the FMERGs. We used 4 LEDs with different wavelength and white light, while Alpern and Kitahara used 30 different monochromatic stimuli. Although each LED provides irradiance with relative steep peak, the relative broad bandwidth in our study might reduce the sensitivity to determine the difference depending on the stimulus wavelength. Using shorter, e.g., 400 nm, and longer, e.g., 700 nm, wavelength stimuli might have increased the sensitivity.

In conclusion, our results showed that the SCE can be determined objectively by the FMERGs. They also indicate that the SCE must be considered in interpreting the results of electrophysiological studies, particularly when focal stimuli are used to assess the macular area. Because the SCE is dependent on the passage of light through the outer segments of the photoreceptors, the FMERGs can be used to examine the alignment and integrity of the photoreceptors of the fovea in different types of macular diseases.

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