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Flicker electroretinograms before and after intravitreal ranibizumab injection in eyes with central retinal vein occlusion

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ABSTRACT.

Purpose: **To compare the amplitudes and implicit times of the flicker electroretinograms before and after an intravitreal injection of ranibizumab (IVR) in eyes with a central retinal vein occlusion (CRVO).**

Methods: **We reviewed the medical records of 15 consecutive patients who had macular oedema secondary to CRVO and had received an IVR at the Nagoya University Hospital from November 2013 to July 2014. Flicker ERGs were recorded with both the RETeval™ system and a conventional ERG system before the IVR. One month after the IVR, recordings were repeated with only the RETeval™ system.**

Results: **The mean implicit times of the flicker ERGs of the affected eyes recorded with the RETeval™ system were significantly longer than that of the fellow eyes (32.2 ± 2.6 msec versus 28.1 ± 1.2 msec, $p < 0.001$). One month after the IVR, the implicit times of the flicker ERGs of affected eyes were significantly shortened from 32.2 ± 2.6 to 30.6 ± 2.2 msec ($p < 0.001$).**

Conclusions: **The shortening of the implicit times of the flicker ERGs after the IVR indicates an improvement of retinal function after anti-VEGF therapy for CRVO eyes.**

Key words: central retinal vein occlusion – electroretinogram – ranibizumab

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Introduction

It has been shown that the implicit times of the flicker electroretinograms (ERGs) are significantly correlated with the degree of retinal ischaemia in eyes with central retinal vein occlusion (CRVO) (Johnson et al. 1988; Johnson & McPhee 1993; Larsson et al. 1998, 2000; Larsson & Andréasson 2001; Kjeka et al. 2007, 2013; Yasuda et al. 2011). Thus, the degree of retinal ischaemia can be monitored by repeated flicker ERG recordings before and after treatment.

A flicker ERG recording system has recently been developed (RETeval™ LKC Technologies, Gaithersburg, MD) that use adhesive skin electrodes as the active electrodes. This system makes it possible to record ERGs less invasively than the conventional ERG systems with contact lens electrodes.

An intravitreal injection of anti-VEGF agents has been widely used to treat the macular oedema due to CRVO (Rosenfeld et al. 2005; Iturralde et al. 2006; Kriechbaum et al. 2007; Campochiaro et al. 2008, 2011, 2014; Brown et al. 2010, 2013; Boyer et al. 2012; Heier et al. 2012, 2014; Holz et al. 2013; Huang et al. 2013; Pielen et al. 2013; Korobelnik et al. 2014; Ogura et al. 2014). However, little is known about the alterations of the retinal function after anti-VEGF therapy.

Thus, the purpose of this study was to compare the different components of the flicker ERGs before and after an intravitreal injection of ranibizumab (IVR) in eyes with a CRVO.

Patients and Methods

The procedures used in this study conformed to the tenets of the World Medical Association's Declaration of Helsinki and were approved by the Nagoya University Hospital Ethics Review Board. An informed consent was obtained from all of the patients after an explanation of the purpose of the study and possible complications.

Patients

We reviewed the medical records of 15 consecutive patients who had macular oedema secondary to a CRVO and had received an IVR at the Nagoya University Hospital from November 2013 to July 2014. Patients with diabetic retinopathy were excluded.

Flicker electroretinograms (ERGs)

Flicker ERGs were recorded with both the RETeval™ system (LKC Technologies, Gaithersburg, MD) and a conventional ERG system (UTAS Visual Testing System with SunBurst™ Ganzfeld; LKC Technologies, Gaithersburg,

MD) before the IVR. One month after the IVR, recordings were repeated with only the RETeval™ system. The rate of stimulation was 28.3-Hz with the RETeval™ system and 30-Hz with the conventional system. Before the ERG recordings, the pupils were dilated by topical 0.5% tropicamide and 0.5% phenylephrine (Mydrin-P, Santen Co., Osaka, Japan) applied over a period of 20 min. The conventional ERGs were elicited with stimuli from a Ganzfeld dome and were recorded with a Burian–Allen bipolar contact lens electrode. The flicker ERGs were elicited by white stimuli of 3 cd-second/m^2 on a white background of 30 cd/m^2 in both ERG systems. Stimuli were obtained from light-emitting diodes (LED) with the RETeval™ and a xenon light source with the conventional system.

Best-corrected visual acuity

The best-corrected visual acuity (BCVA) was measured by a standard Japanese decimal visual acuity chart at 5 m. The decimal values were converted to the logarithm of the minimum angle of resolution (logMAR) units for statistical analyses.

Foveal thickness

The foveal thickness was determined by optical coherence tomography (OCT; Cirrus model; Carl Zeiss Meditec, Dublin, CA). After the patients' pupils were fully dilated with 0.5% tropicamide and 0.5% phenylephrine (Mydrin-P, Santen Co), 6 mm vertical and horizontal scans were made through the fovea. The average foveal thickness of the vertical and horizontal scans was used as the foveal thickness. We used a manual method to place the cursors on the OCT images to measure the foveal thickness because it has been reported that the automatic measurements of the foveal thickness often failed to identify the outer border of the neural retina.

Statistical analyses

The correlations in the amplitudes and implicit times of the flicker ERGs recorded with the RETeval™ system and the conventional ERG system were determined by the Pearson correlation coefficient. The significance of the differences in the amplitudes and implicit times between before and after IVR was determined by paired *t*-tests. A commercially available software (SPSS v. 17.0J for Windows; SPSS Inc., Chicago, IL) was used for all statistical analyses. A $p < 0.05$ was considered significant.

Results

Patients

The medical records of 15 eyes of 15 consecutive patients were studied. The characteristics of the 15 eyes are shown in Table 1. There were 10 men and five women whose mean ± SD age was 71.6 ± 10.3 years with a range of 46 to 83 years. The mean duration of the symptoms before the IVR was 6.9 ± 5.3 weeks with a range of 2 to 20 weeks. At the time of the IVR, the mean visual acuity was 0.84 ± 0.56 logMAR units with a range of 0.05 to 2.00 logMAR units. The pre-IVR mean foveal thickness determined by OCT was 663 ± 235 μm with a range of 348 to 1208 μm (Table 1). There was no neovascularization of the angle or on the iris in any patient before the IVR.

One month after the IVR, the mean visual acuity improved to 0.57 ± 0.43 logMAR units. The mean foveal thickness determined by OCT was reduced to 297 ± 50 μm.

Table 1. Characteristics of our patients with CRVO

Number of eyes	15
Age (year)*	71.6 ± 9.7 (46–83)
Sex (man/women)	10/5
Duration of symptoms before ERG examination (weeks)*	6.9 ± 5.3 (2–20)
Pre-IVR photocoagulation (eyes)	0
Pre-IVR visual acuity (logMAR)*	0.84 ± 0.56 (0.05–2.00)
Pre-IVR foveal thickness (μm)*	663 ± 235 (348–1208)
Visual acuity 1 month after the IVR (logMAR)*	0.57 ± 0.43 (0.00–1.30)
Foveal thickness 1 month after the IVR (μm)*	297 ± 50 (246–425)

*Data are expressed as mean ± SD (range).

Correlation between flicker ERGs recorded with RETeval™ system and conventional system before treatment

The mean implicit time of the flicker ERGs recorded before the IVR by the RETeval™ system was 32.2 ± 2.6 msec, which was slightly longer than that recorded by the conventional system at 31.4 ± 3.1 msec ($p = 0.46$). The implicit times recorded by the two systems were significantly correlated ($r = 0.89$, $p < 0.001$, Pearson correlation coefficient; Fig. 1A). The amplitudes of the flicker ERGs recorded by the two systems were also significantly correlated ($r = 0.66$, $p = 0.008$, Pearson correlation coefficient; Fig. 1B).

Comparisons of flicker ERG components between affected and fellow eyes recorded with RETeval™ system before treatment

The mean implicit times of the flicker ERGs of the affected eyes recorded

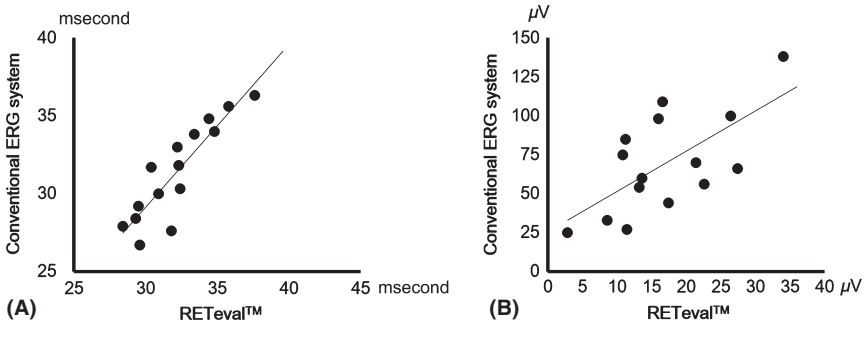

Fig. 1. Correlation between flicker ERGs recorded with RETeval™ system and conventional system before intravitreal injection of ranibizumab (IVR). (A) The implicit times of flicker ERGs recorded before the IVR with the RETeval™ system were significantly correlated to those recorded by the conventional system ($r = 0.89$, $p < 0.001$, Pearson correlation coefficient). (B) The amplitudes of flicker ERGs recorded by the two systems are also significantly correlated ($r = 0.66$, $p = 0.008$, Pearson correlation coefficient).

with the RETeval™ system were significantly longer than that of the fellow eyes (32.2 ± 2.6 msec versus 28.1 ± 1.2 msec, p < 0.001, student's *t*-test). The difference of the amplitudes of the flicker ERGs between the affected eyes and the fellow eyes was not significant (16.9 ± 8.2 μV versus 20.4 ± 7.3 μV, p = 0.22).

Comparisons of flicker ERG components before and after IVR

One month after the IVR, the implicit times of the flicker ERGs recorded with the RETeval™ system were significantly shortened from 32.2 ± 2.6 msec to 30.6 ± 2.2 msec (p < 0.001; paired *t*-test; Figs 2A and 3). The difference in the amplitudes of the flicker ERGs before and after the IVR was not significant (16.9 ± 8.2 μV versus 17.3 ± 6.6 μV; paired *t*-test; p = 0.67; Fig. 2B).

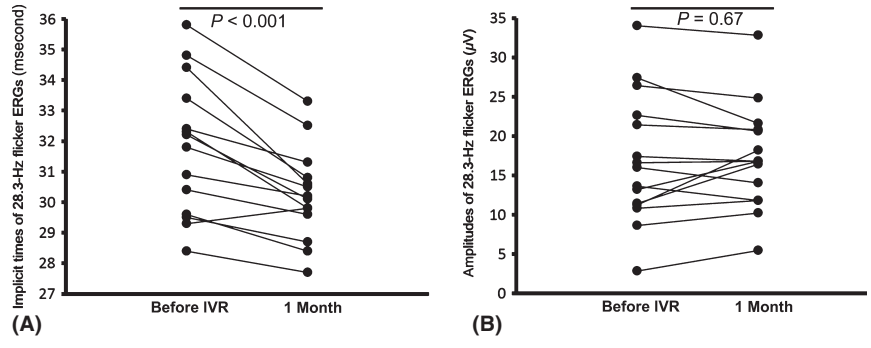

Fig. 2. Amplitudes and implicit times of the flicker ERGs before and after IVR recorded with the RETeval™ system. (A) One month after the IVR, the implicit times of the flicker ERGs recorded by the RETeval™ system were significantly shortened from 32.2 ± 2.6 msec to 30.6 ± 2.2 msec (p < 0.001; paired *t*-test). (B) The difference of the amplitudes of the flicker ERG before and after the IVR was not statistically significant (16.9 ± 8.2 versus 17.3 ± 6.6 μV; paired *t*-test, p = 0.67).

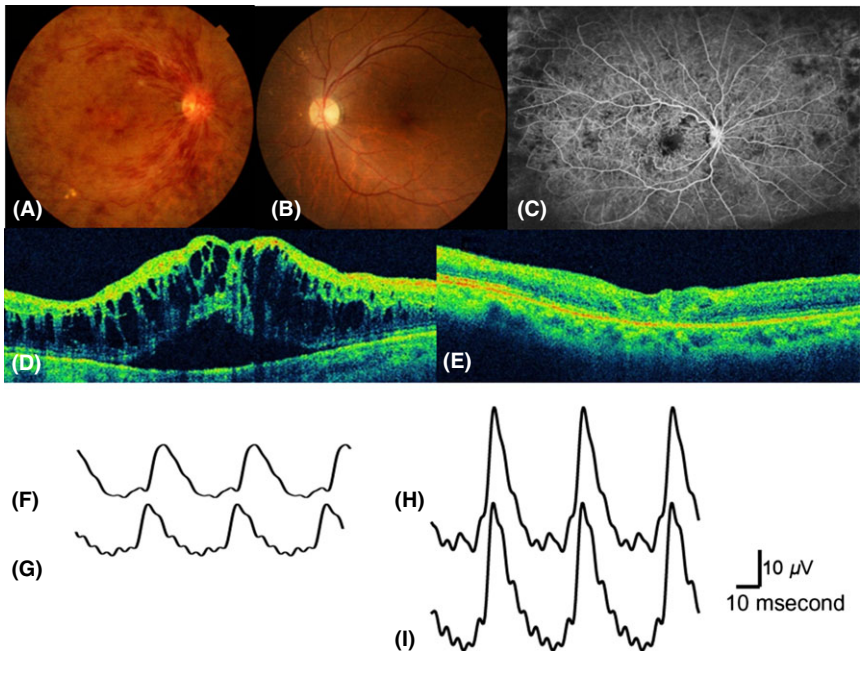

Fig. 3. Example of the changes of the flicker ERGs recorded before and at 1 month after IVR in patient with CRVO (Patient 14, 83-year-old man). Fundus photograph of the affected eye (A), fellow eye (B) and fluorescein angiogram before the treatment (C) are shown. Horizontal foveal OCT scan before the treatment (D) and 1 month after the treatment (E) is shown. The foveal thickness decreased from 593 to 246 μm. The flicker ERGs of the affected eye recorded by the RETeval™ system before (F) and 1 month after the treatment (G) are shown. The implicit time shortened from 34.4 msec to 30.6 msec. The flicker ERGs of the fellow eye recorded by the RETeval™ system before (H) the treatment and 1 month after the treatment (I) are shown.

Discussion

Our results showed that the implicit times of the flicker ERGs were significantly reduced after the IVR which would suggest a recovery of retinal function. This seems reasonable because there is a positive feedback loop in eyes with a CRVO; the retinal ischaemia induced by the CRVO results in the expression of VEGF and the higher levels of VEGF promote the progression of retinal ischaemia by increasing leukostasis (Miyamoto et al. 2000; Ishida et al. 2003). The blockage of VEGF by the ranibizumab appears to disrupt this positive feedback loop (Campochiaro et al. 2013; Sophie et al. 2013).

It has also been reported that the retinal non-perfused areas detected by fluorescein angiography were decreased after 3 monthly ranibizumab injections (Sophie et al. 2013). Thus, the disruption of the positive feedback loop and decrease in the non-perfused areas may account for the shortening of the implicit times of the flicker ERGs after the IVR. These changes might then be associated with the recovery of the retina from the ischaemia.

Neubauer et al. performed semi-quantitative measurements of retinal non-perfusion areas using the ultra wide-field scanning laser ophthalmoscope before and 1 month after the intravitreal injection of bevacizumab (IVB) in eyes with diabetic retinopathy (Neubauer et al. 2007). They reported that that the mean area of the peripheral ischaemia significantly decreased 1 month after the IVB therapy. They suggested that the anti-VEGF therapy probably improved the peripheral retinal ischaemia in the short term. These findings support our results although the pathology of CRVO is different from that of diabetic retinopathy (Terui et al. 2011).

High levels of VEGF result in retinal oedema not only in the macula but also in the entire retina. Because the oedema causes malfunctioning of the retina, another possibility for the shortened implicit times of the flicker ERGs after the IVR is a reduction of retinal oedema after the IVR. This

would then lead to an improvement of retinal function. However, further studies are necessary to determine the mechanism for the improvement of the implicit times of the flicker ERGs after IVR.

In the natural course of CRVO, neovascular glaucoma usually occurs in the first 8 months (Hayreh et al. 1983); however, it has been reported that anti-VEGF therapy delays the neovascular complications (Wykoff et al. 2014). The Rubeosis Anti-VEGF (RAVE) Trial Study reported that the risk of neovascular complications in the CRVO eyes with severe ischaemia was not completely ameliorated by anit-VEGF injections. Thus, longer follow-up periods are needed to determine the alterations of the retinal function after anti-VEGF therapy.

During the course of CRVO in eyes receiving anti-VEGF therapy, we need to evaluate the degree of retinal ischaemia for a long period of time to avoid the neovascular glaucoma. It would be helpful to estimate the degree of ischaemia by repeated flicker ERG recordings. Although recording the ERGs by the conventional method is relatively non-invasive, a contact lens electrode must still be inserted on an anesthetized eye. Experience has shown that contact lens electrodes can cause corneal abrasions which can be painful with a potential of causing infections of the cornea. Thus, an ERG recording system that is less invasive would be helpful for repeated monitoring of the flicker ERGs.

One limitation of this study is that we need more data to confirm that the RETeval™ system can be used as an alternative for the conventional ERG system to record the flicker ERGs. Thus, further studies are needed to confirm the validity of the flicker ERGs recorded by the RETeval™ system.

In conclusion, the implicit times of the flicker ERGs were significantly shortened after IVR indicating an improvement of retinal function.

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