Significant Correlation between Electroretinogram Parameters and Ocular Vascular Endothelial Growth Factor Concentration in Central Retinal Vein Occlusion Eyes

Shunsuke Yasuda, Shu Kachi, Mineo Kondo, Hiroaki Ushida, Ruka Uetani, Takayuki Terui, Chang-Hua Piao, and Hiroko Terasaki

PURPOSE. Central retinal vein occlusion (CRVO) leads to retinal ischemia, which then induces an upregulation of vascular endothelial growth factor (VEGF). The aim of this study was to determine whether a significant correlation exists between the ocular VEGF levels and the amplitudes and implicit times of different components of the electroretinogram (ERG) in eyes with a CRVO.

METHODS. The medical records of the 20 consecutive patients who had macular edema secondary to CRVO and were examined at the Nagoya University Hospital from November 2008 to February 2010 were reviewed. Because all the patients were scheduled to receive an intravitreal injection of bevacizumab (IVB), it was possible to collect samples of the aqueous humor before the IVB. The correlation between the different components of the ERGs and the VEGF concentration in the aqueous was determined.

RESULTS. The mean VEGF concentration of the aqueous humor was 416 pg/mL with a range of 100-1260 pg/mL. The b/a ratio of the single flash ERGs (P = 0.049; r = −0.45), implicit times of the cone a-wave (P = 0.028; r = 0.50), cone b-wave (P = 0.0059; r = 0.65), and 30 Hz flicker ERGs (P = 0.0058; r = 0.65) were significantly correlated with the VEGF concentration in the aqueous.

CONCLUSIONS. The significant correlations between the different components of the ERGs and the aqueous VEGF concentration indicate that full-field ERGs can be used to detect the CRVO patients at a high risk of developing neovascularization of the iris. (Invest Ophthalmol Vis Sci. 2011;52:5737–5742) DOI: 10.1167/iovs.10-6923

A central retinal vein occlusion (CRVO) is a common retinal vascular disorder that is associated with an increase in the production of endothelial growth factor (VEGF) from the ischemic retina.1,2 The increased levels of VEGF is believed to lead to neovascularization of the iris (NVI) and/or anterior chamber angle and then progress to neovascular glaucoma (NVG).3 In the Central Vein Occlusion Study, 16% of the eyes with a CRVO developed NVI.4 It is important to determine the degree of retinal ischemia in patients with CRVO because the NVG can be prevented by panretinal laser photocoagulation.

Electroretinography (ERG) is a noninvasive method that can be used to determine the degree of retinal ischemia, and this test can be performed repeatedly during the course of the CRVO.5–7 The results of several studies have demonstrated that different components of the full-field ERGs, e.g., amplitudes of the b-wave, b/a-wave amplitude ratio, and the implicit times of the 30 Hz flicker ERGs, can be helpful in distinguishing the ischemic type from nonischemic type of CRVO.5–7 However, there is no report that showed whether these ERG parameters were significantly correlated with the intraocular VEGF level.

Macular edema is one of the major causes of the visual reduction in eyes with a CRVO, and an intravitreal injection of bevacizumab (IVB) has been shown to be effective in reducing the macular edema.26–28 Because an anterior chamber paracentesis is usually performed before the IVB, this has allowed investigators to collect samples of the aqueous to measure the concentration of different bioactive proteins in the aqueous.

Thus, the purpose of this study was to determine whether there were significant correlations between the values of the different components of the full-field ERGs and the concentrations of VEGF in the aqueous of 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. The IVB, collection of aqueous humor, and VEGF measurements were performed after obtaining approval of Nagoya University Hospital Ethics Review Board and a written informed consent from each patient.

Patients

We reviewed the medical records of the 20 consecutive patients who received IVB for macular edema secondary to CRVO at the Nagoya University Hospital from November 2008 to February 2010. Patients with diabetic retinopathy were excluded from this study.

Electroretinograms

The ERGs were elicited with stimuli from a Ganzfeld dome and were recorded with a Burian-Allen bipolar contact lens electrode. The eyes were dark-adapted for 30 minutes, and a rod response was elicited by blue light at an intensity of 5.2 × 10^3 candelas (cd)-second/m^2. A mixed cone-rod ERG was elicited by a white flash of 44.2 cd-second/m^2, and the cone ERGs and 30-Hz flicker ERGs were elicited by white stimuli of 4 cd-second/m^2 and 0.9 cd-second/m^2, respectively. The
Pre-IVB photocoagulation (eyes) was performed on these two patients after the bevacizumab injection.

**Comparisons of ERG Components between Affected and Fellow Eyes**

The amplitudes and implicit times of the rod, cone, single bright flash, and 30 Hz flicker ERGs of the affected and the fellow eyes are shown in Table 2. The amplitudes of the rod b-wave, cone a- and b-waves, single flash a- and b-waves, and the 30 Hz flicker of the affected eyes were significantly smaller than that of the fellow eyes. The implicit times of all these components of the affected eyes were also significantly longer than that of the fellow eyes except for the single flash b-wave.

**Correlation between ERG Components and Concentration of VEGF in Aqueous Humor**

The coefficients of correlation between ERG components and the VEGF concentration in the aqueous humor were determined by Spearman’s rank correlations (Table 3). The b/a ratio of single flash ERGs (P = 0.049; ρ = −0.45), implicit times of the cone a-wave (P = 0.028; ρ = 0.50), cone b-wave (P = 0.0059; ρ = 0.63), and 30 Hz flicker ERG (P = 0.0058; ρ = 0.63) were significantly correlated with the VEGF concentration in the aqueous (Fig. 1).

**Ocular VEGF Level in CRVO Eyes with Shorter or Longer 30 Hz Flicker ERG Implicit Times**

Two studies have reported that eyes with a CRVO had a higher risk of NVG if the implicit times of the 30 Hz flicker ERGs were >37 msec or >35 msec.21,24 Therefore, we divided the eyes according to whether the implicit time was >37.1 msec or ≤35.1 msec. The VEGF concentration of the five eyes with implicit times >37.1 msec was more than three times higher than that of the 15 eyes with implicit time ≤37.1 msec (909 pg/mL vs. 252 pg/mL; P = 0.01, Mann-Whitney U-test).

When we used a cutoff value of 35 msec, the mean ocular VEGF concentration in the eyes with implicit time of >35 msec was more than two times higher than that of the eyes with implicit time ≤35 msec (668 pg/mL vs. 248 pg/mL; P = 0.015, Mann-Whitney U-test; Figure 2).

**Relationship between Ocular VEGF Level and Visual Acuity**

Because it is known that a low visual acuity is a predictive factor for the development of NVI, we examined whether there was a significant correlation between visual acuity and the VEGF level, and whether there is any difference in VEGF level.5,33 Our analyses showed that the correlation between visual acuity and the VEGF level was not significant (P = 0.15, ρ = 0.33, Spearman’s rank test; Fig. 3).

The mean VEGF concentration was 877 pg/mL in the eyes with a visual acuity < 20/400, which was higher than the 335 pg/mL of the >20/400 group. However, as there were only three eyes in the <20/400 group, statistical significance was not obtained (Fig. 3). When we used a cutoff value of 20/200, the mean VEGF concentration of five eyes with visual acuity ≤20/200 was 649 pg/mL, which was not significantly higher than 339 pg/mL of the >20/200 group (P = 0.13, Mann-Whitney U-test).

Although the pupillary reflexes can be a predictive factor for NVI, we did not perform pupillary testing in any of the patients.54,55

**DISCUSSION**

Our results showed that the concentration of VEGF in the aqueous of eyes with a CRVO was significantly correlated with

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**Measurement of VEGF Level in Aqueous by ELISA**

The aqueous samples were stored at −80°C until use. The concentration of VEGF was measured by enzyme-linked immunosorbent assay using a commercially available kit (Quantikine; R&D Systems, Minneapolis, MN), which measures both human VEGF121 and VEGF165.32

**Statistical Analyses**

The correlation between the different components of the ERG and the VEGF level in the aqueous humor was determined with the Spearman’s rank test. We classified the 20 eyes into two groups according to the implicit time of the 30 Hz flicker, and the differences in the VEGF level of aqueous humor between the two groups was determined with the nonparametric Mann-Whitney U-test. Commercially available software (SPSS v. 17.0J for Windows; SPSS Inc., Chicago, IL) was used for all statistical analyses. P < 0.05 was considered significant.

**RESULTS**

**Demographics and Ocular VEGF Concentrations of Patients**

Twenty eyes of 20 consecutive patients were studied. There were 13 men and 7 women whose mean age was 63.9 years with a range of 26–78 years. The mean duration of the symptoms before the IVB was 16.3 weeks (3–54 weeks). At the time of the IVB, the mean visual acuity was 0.84 logarithm of the minimum angle of resolution (logMAR) units with a range of 469–1625 logMAR units. The mean foveal thickness determined by optical coherence tomography (OCT) was 720 μm with a range of 469–1625 μm (Table 1).

Before the IVB, the mean VEGF concentration in the aqueous humor was 416 pg/mL using a 30-gauge needle inserted through the sclera 3.5 mm from the limbus. Antibiotics drops were given for three days after the injection.

<table>
<thead>
<tr>
<th>TABLE 1. Characteristics and the VEGF Concentration of Our Patients with CRVO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of eyes</td>
</tr>
<tr>
<td>Age (year)*</td>
</tr>
<tr>
<td>Sex (male/female)</td>
</tr>
<tr>
<td>Duration of symptoms before ERG examination (weeks)*</td>
</tr>
<tr>
<td>Pre-IVB photocoagulation (eyes)</td>
</tr>
<tr>
<td>Pre-IVB visual acuity (logMAR)*</td>
</tr>
<tr>
<td>Pre-IVB foveal thickness (μm)*</td>
</tr>
<tr>
<td>Pre-IVB aqueous level of VEGF (pg/mL)*</td>
</tr>
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</table>

* Data were expressed as mean ± SEM (range).

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**Intravitreal Injection of Bevacizumab and Collection of Aqueous Humor**

The eyes were anesthetized with topical 1% tetracaine, and the fornices were irrigated with 10% providone-iodine. A mean volume of 0.1 mL of aqueous humor was collected by anterior chamber paracentesis with a 27-gauge needle attached to a 1 mL syringe.30,31 The 27-gauge needle was used for the anterior chamber tap because this is the general protocol in our department.

Each patient then received an intravitreal injection of 1.25 mg/0.05 mL of bevacizumab using a 30-gauge needle inserted through the sclera 3.5 mm from the limbus. Antibiotics drops were given for three days after the injection.

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**Correlation between Ocular VEGF Level and Visual Acuity**

Because it is known that a low visual acuity is a predictive factor for the development of NVI, we examined whether there was a significant correlation between visual acuity and the VEGF level, and whether there is any difference in VEGF level.5,33 Our analyses showed that the correlation between visual acuity and the VEGF level was not significant (P = 0.15, ρ = 0.33, Spearman’s rank test; Fig. 3).

The mean VEGF concentration was 877 pg/mL in the eyes with a visual acuity < 20/400, which was higher than the 335 pg/mL of the >20/400 group. However, as there were only three eyes in the <20/400 group, statistical significance was not obtained (Fig. 3). When we used a cutoff value of 20/200, the mean VEGF concentration of five eyes with visual acuity ≤20/200 was 649 pg/mL, which was not significantly higher than 339 pg/mL of the >20/200 group (P = 0.13, Mann-Whitney U-test).

Although the pupillary reflexes can be a predictive factor for NVI, we did not perform pupillary testing in any of the patients.54,55

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**Discussion**

Our results showed that the concentration of VEGF in the aqueous of eyes with a CRVO was significantly correlated with...
TABLE 3. Correlation of ERG Components to Ocular VEGF in CRVO Eyes

<table>
<thead>
<tr>
<th>VEGF Concentration</th>
<th>Rod b-wave</th>
<th>Single flash a-wave</th>
<th>Single flash b-wave</th>
<th>Cone a-wave (average ± SEM) mV</th>
<th>Cone b-wave</th>
<th>30 Hz flicker</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15.4–153.8 (73.8 ± 9.2)</td>
<td>15.8–507.7 (328.5 ± 22.9)</td>
<td>169.2–646.2 (456.2 ± 31.2)</td>
<td>7.7–38.5 (27.0 ± 2.3)</td>
<td>30.8–130.8 (76.2 ± 6.8)</td>
<td>5.8–65.5 (20.5 ± 2.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>46.2–169.2 (99 ± 7.4)</td>
<td>215.4–538.5 (336.2 ± 16.7)</td>
<td>261.5–615.4 (464.6 ± 25.0)</td>
<td>23.1–53.8 (33.1 ± 2.1)</td>
<td>53.8–146.1 (86.2 ± 6.2)</td>
<td>9.6–65.5 (25.1 ± 2.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

TABLE 2. ERG Components of the Affected Eyes and the Fellow Eyes

<table>
<thead>
<tr>
<th>ERG Components</th>
<th>Amplitude of the Affected Eyes (average ± SEM) µV</th>
<th>Amplitude of the Fellow Eyes (average ± SEM) µV</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rod b-wave</td>
<td>15.4–153.8 (73.8 ± 9.2)</td>
<td>61.5–119.2 (92.1 ± 3.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Single flash a-wave</td>
<td>15.8–507.7 (328.5 ± 22.9)</td>
<td>10.2–16.2 (14.0 ± 0.39)</td>
<td>0.0246</td>
</tr>
<tr>
<td>Single flash b-wave</td>
<td>169.2–646.2 (456.2 ± 31.2)</td>
<td>44.4–60.0 (53.7 ± 1.2)</td>
<td>0.2791</td>
</tr>
<tr>
<td>Cone a-wave</td>
<td>7.7–38.5 (27.0 ± 2.3)</td>
<td>15.2–20.3 (17.8 ± 0.32)</td>
<td>0.0153</td>
</tr>
<tr>
<td>Cone b-wave</td>
<td>30.8–130.8 (76.2 ± 6.8)</td>
<td>51.0–46.6 (37.0 ± 0.90)</td>
<td>0.0002</td>
</tr>
<tr>
<td>30 Hz flicker</td>
<td>5.8–65.5 (20.5 ± 2.2)</td>
<td>29.0–40.4 (32.2 ± 0.54)</td>
<td>0.0015</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>0.0002</td>
<td>0.0001</td>
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</table>

Correlation of ERG Components and VEGF Concentration

<table>
<thead>
<tr>
<th>ERG Components</th>
<th>Amplitude (average ± SEM) µV</th>
<th>p</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rod b-wave</td>
<td>15.4–153.8 (73.8 ± 9.2)</td>
<td>0.32</td>
<td>0.17</td>
</tr>
<tr>
<td>Single flash a-wave</td>
<td>15.8–507.7 (328.5 ± 22.9)</td>
<td>0.27</td>
<td>0.25</td>
</tr>
<tr>
<td>Single flash b-wave</td>
<td>169.2–646.2 (456.2 ± 31.2)</td>
<td>0.21</td>
<td>0.36</td>
</tr>
<tr>
<td>Cone a-wave</td>
<td>7.7–38.5 (27.0 ± 2.3)</td>
<td>0.25</td>
<td>0.28</td>
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<tr>
<td>Cone b-wave</td>
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<td>0.0059</td>
</tr>
<tr>
<td>30 Hz flicker</td>
<td>5.8–65.5 (20.5 ± 2.2)</td>
<td>0.63</td>
<td>0.0058</td>
</tr>
</tbody>
</table>

Table 3.

Correlation of ERG Components to Ocular VEGF in CRVO Eyes

<table>
<thead>
<tr>
<th>ERG Components</th>
<th>Implicit Time of the Affected Eyes (average ± SEM) msec</th>
<th>Implicit Time of the Fellow Eyes (average ± SEM) msec</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rod b-wave</td>
<td>61.5–119.2 (92.1 ± 3.4)</td>
<td>69.2–115.4 (89.2 ± 2.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Single flash a-wave</td>
<td>10.2–16.2 (14.0 ± 0.39)</td>
<td>10.4–14.6 (12.7 ± 0.27)</td>
<td>0.0246</td>
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<td>Single flash b-wave</td>
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<td>30 Hz flicker</td>
<td>29.0–42.2 (34.3 ± 0.84)</td>
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<td></td>
<td>0.0001</td>
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</tr>
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</table>

The b/a ratio and with the implicit times of the cone a-wave, b-wave, and 30 Hz flicker ERGs. There have been eight studies that report that the amplitudes of the a- and b-waves and the b/a ratio are good indicators that can differentiate the ischemic type from the nonischemic type of CRVO.6–8,10,13,16,17,19 In addition, the implicit times of the b-wave and 30 Hz flicker ERGs are good predictors for the development of ruberosis in eyes with CRVO.14,16,23

The longer implicit times of the 30 Hz flicker ERGs were significantly associated with the development of NVG. Larsson et al.21 reported that the mean implicit times of the 30 Hz flicker ERGs in all CRVO eyes that developed ruberosis was >37.1 msec, and all eyes that did not develop ruberosis was <37 msec. Kjeka et al.24 reported that implicit times of the 30 Hz flicker ERG >35 msec in the CRVO eyes were associated with the development of ocular neovascularization. Our study showed that the VEGF concentration was about two times higher in eyes with implicit times of the 30 Hz flicker ERGs >35 msec than in eyes with <35 msec (P = 0.015). In addition, the VEGF concentration was three times higher in eyes with implicit times of the 30 Hz flicker ERGs >37 msec than in eyes with <37 msec (P = 0.01). Interestingly, the implicit time of all three eyes with VEGF concentration of >1000 mg/mL was 37.6, 40.6, and 42.2 msec. Thus, our results are consistent with the earlier results. In addition, Johnson et al.14 reported that the implicit times of the flicker ERGs were the most predictive ERG parameter for NVI and their cutoff value was 40 msec. Consistent with their report, the two eyes with implicit times >40 msec in our study had high levels of VEGF, viz., 1170 and 1210 pg/mL, although we could not perform statistical analysis because there were only 2 eyes. The differences in the cutoff time are partially due to the different stimulus luminances used in these studies, because the implicit time is very sensitive to stimulus luminances. However, these studies are all in agreement that the 30 Hz flicker ERG implicit time is significantly associated with the development of NV in eyes with CRVO.

Additionally, the mean VEGF concentration of our eyes with prolonged implicit times (668 pg/mL in eyes with >35 msec) was higher than the mean concentration of six PDR eyes with NVI reported by Matsuyama et al.6 (630 pg/mL, measured with the same type of ELISA kit as we used).26 These findings also suggested a high risk of NVI in eyes with prolonged implicit times, although CRVO eyes can be different from PDR eyes in the probability of developing NVI. It has been reported that some fellow eyes have prolonged implicit times even without any abnormality of the fundus.27 Thus, we have compared the ERGs recorded from the fellow eyes to 20 eyes of age-matched normal controls, and the differences in the ERG parameters were not significant. However, as there were some fellow eyes that had shown prolonged implicit time or low amplitude ERGs, careful follow-ups for these eyes might be needed.

There are two limitations in this study. The first limitation was that the ERG recording conditions were not exactly the same as that recommended by the International Society for Clinical Electrophysiology of Vision. This may be also why the implicit time of the maximum flash scotopic ERG was not statistically delayed in the affected versus fellow eyes. However, these differences should not invalidate our findings. The second limitation was that none of the eyes developed NVI.
during the follow-up period. However, because laser treat-
ment was performed to all the eyes with nonperfusion area,
and bevacizumab was injected into all eyes, we cannot tell
which eyes would have developed NVI during their natural
course.

In conclusion, the VEGF concentration in the aqueous of
eyes with a CRVO was significantly correlated with the
implicit time of the 30 Hz flicker ERGs, cone a- and b-waves,
and the b/a ratio of single flash ERG. When the 30 Hz flicker
ERG implicit time is $>35$ msec, the ocular VEGF level was
more than two times higher than that of eyes with implicit
time $<35$ msec. Our findings indicate that the full-field ERGs
can be used to detect eyes that are at a high risk of develop-
ing NVI.

**Figure 1.** Relationship between the values of the ERG components and the VEGF concentration in the aqueous humor. (A) The b/a-wave amplitude ratios are correlated with ocular VEGF concentration ($P = 0.049$, $\rho = -0.45$). (B) Implicit times of cone a-wave are correlated with ocular VEGF concentration ($P = 0.028$, $\rho = 0.50$). (C) Implicit times of cone b-wave are correlated with ocular VEGF concentration ($P = 0.0059$, $\rho = 0.65$). (D) Implicit times of 30 Hz flicker ERGs are correlated with ocular VEGF concentration ($P = 0.0058$, $\rho = 0.63$).

**Figure 2.** Ocular VEGF concentrations in eyes with long and short 30 Hz flicker ERG implicit times. (A) Comparison of ocular VEGF levels in eyes with implicit time of 35 msec or longer and in eyes with implicit time of shorter than 35 msec. The ocular VEGF level of the $\geq 35$ msec group is significantly higher than that of the $<35$ msec group ($P = 0.02$). (B) Comparison of ocular VEGF levels in eyes with implicit time of 37.1 msec or longer and in eyes with implicit time of shorter than 37 msec. The ocular VEGF level in the $\geq 37.1$ msec group is significantly higher than that of the $<37$ msec group ($P = 0.01$). The top and bottom ends of the box indicate the 25 and 75 percentiles, line in the box indicates the median, and the top and bottom ends of the error bars indicate the 2.5 and 97.5 percentiles.
Correlation of ERG Components to Ocular VEGF in CRVO Eyes

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References


