Correlation between Focal Macular Electroretinograms and Angiographic Findings after Photodynamic Therapy

Kohei Ishikawa, Mineo Kondo, Yasuki Ito, Masato Kikuchi, Hiroaki Nishibara, Chang-Hua Piao, Tadasu Sugita, and Hiroko Terasaki

Purpose. It is known that the amplitudes of the multifocal electroretinograms are generally reduced soon after photodynamic therapy (PDT). The purpose of this study was to determine whether this amplitude reduction correlates with the changes in macular thickness or with changes in choroidal circulation.

Methods. Thirty-seven eyes that were successfully treated by PDT were studied. Focal macular electroretinograms (fmERGs) and optical coherence tomography were performed before and 1 week, 1 month, and 3 months after PDT. Indocyanine green angiography was performed before and 3 months after PDT. The indocyanine green angiographic findings were classified into two groups: group A, with indistinct hypofluorescence at the site of the PDT, and group B, with well-defined hypofluorescence borders coinciding with the site of the PDT.

Results. The mean amplitudes of the fmERGs were significantly reduced at 1 week after PDT (P < 0.05). The correlations between the changes in the amplitude of the fmERG and the changes in macular thickness were not significant. Sixteen (43%) of the study eyes were classified into group A and 21 (57%) into group B by indocyanine green angiography. The mean ratio of the fmERG b-wave 1 week after PDT to that before PDT was 1.14 ± 0.62 in group A and 0.65 ± 0.29 in group B. This difference was statistically significant (P < 0.01).

Conclusions. One of the possibilities that could explain the reduction in the amplitude of the fmERGs soon after PDT is the reduction in choroidal circulation caused by the PDT. (Invest Ophtalmol Vis Sci. 2007;48:2254–2259) DOI:10.1167/iovs.06-1277

Photodynamic therapy (PDT) with verteporfin is one option for the management of choroidal neovascularization (CNV) associated with age-related macular degeneration (AMD) and other retinal diseases.1–6 In this treatment, a nonthermal laser associated with age-related macular degeneration (AMD) and myopic CNV.1–4

Although PDT has been generally regarded as a safe procedure, acute visual disturbances have been reported in a small number of cases relatively early after PDT. For example, in the VIP study,4 10 (4.4%) of 225 patients reported a severe visual decrease within 7 days after PDT, and 3 (0.7%) of 402 patients had a similar decrease in the TAP study.2 A review of these cases showed that the acute visual loss after PDT was associated with abnormal findings including serous retinal detachment, subretinal hemorrhage, abnormal choroidal perfusion, and retinal color changes. However, four of these patients did not show any abnormality by ophthalmoscopy and fluorescein angiography.

To study the changes in macular function after PDT objectively, multifocal electroretinograms (mfERGs) have been recorded by many researchers before and after PDT.8–13 In some of these studies,9,10,12 it was reported that the amplitudes of mfERGs were reduced within 2 weeks after PDT and then recovered gradually over 3 months. These results suggest that macular function may be transiently impaired after PDT. However, the exact mechanism causing the retinal dysfunction has not been determined, although several possibilities have been proposed: ischemic changes induced by altered choroidal circulation,12,14 acute inflammatory response associated with vascular leakage,12,14 and damage to the photoreceptors or bipolar cells by the PDT.15

The purpose of this study was to determine the cause of depressed retinal function that occurs soon after PDT. To answer this question, we examined macular thickness by optical coherence tomography (OCT) and choroidal circulation by indocyanine green angiography (ICGA) and compared them to the focal macular electroretinograms (fmERGs). The reduction in the amplitude of the fmERGs after PDT was significantly greater in patients with a well-defined choroidal hypofluorescence border coinciding with the site of the PDT than in patients with indistinct choroidal hypofluorescence. This suggests that the transient amplitude decrease in fmERGs after PDT is probably related to the decreased choroidal circulation caused by PDT.

Methods

Patients

The medical records of 37 eyes of 36 patients (25 men, 11 women, mean age 72.2 ± 8.7 years, mean ± SD), who underwent PDT at the Nagoya University Hospital, were examined, retrospectively. Patients with subfoveal CNV caused by AMD and those with polyoidal choroidal vasculopathy (PCV) were studied. Patients who had CNV lesions that were due to other retinal diseases or who had other ophthalmic diseases were excluded. Of the 37 eyes, 22 (59%) had AMD and 15 (41%) had PCV. We measured the greatest linear dimension (GLD) before PDT by fluorescein angiography, according to the guidelines of the TAP (Treatment of Age-Related Macular Degeneration with Photo-
VIP3,4 studies. Patients received 6 mg/m² intravenous verteporfin.

The mean of the GLD of the lesion was 3621 ± 1320 μm (± SD; range, 1676–6479).

The research was conducted in accordance with the institutional guidelines of Nagoya University, and the procedures used conformed to the tenets of the World Medical Association’s Declaration of Helsinki. An informed consent had been obtained for the examinations and PDT from each of the patients after they were provided sufficient information on the procedures to be used.

Visual Acuity

The standard Japanese chart was used to measure visual acuity, and the results were converted to Snellen visual acuity and to the logarithm of the minimal angle of resolution (logMAR) for statistical analyses.

Photodynamic Therapy

PDT was performed according to the guidelines of the TAP1,2 and VIP3,4 studies. Patients received 6 mg/m² intravenous verteporfin (Visudyne; Novartis, Basel, Switzerland) over a 10-minute period. Fifteen minutes after the beginning of the infusion, a 689-nm diode laser was used to deliver 600 mW/cm² for at least 83 seconds, to produce an energy dose of 50 J/cm². The diameter of the laser spot was calculated to be 1000 μm larger than the GLD of the CNV lesion. After treatment, patients were given protective spectacles, and they were instructed to avoid direct sunlight or strong light for 3 days.

Focal Macular Electroretinograms

fmERGs were recorded before and 1 week, 1 month, and 3 months after PDT. Our system for eliciting and recording fmERGs has been described in detail.15,16 Briefly, an infrared fundus camera, equipped with a stimulus light, background illumination, and fixation target, was used. The image from the camera was fed to a television monitor, and the examiner used the images on the monitor to maintain the stimulus with a stimulus light, background illumination, and fixation target, was used. The image from the camera was fed to a television monitor, and the examiner used the images on the monitor to maintain the stimulus centered on the fovea. The size of the stimulus spot was 15°, and the background light was delivered to the eye from the fundus camera at a visual angle of 45°. Additional background illumination outside the central 45° produced a homogeneous background for nearly the entire visual field. The luminances of the white stimulus light and background light were 29.46 and 2.89 cd/m², respectively. These luminances were measured at the corneal surface and then converted to the retinal surface. A Burian-Allen bipolar contact lens electrode was used to pick-up the fmERGs. This contact lens electrode system allowed not only low electrical noise but also permitted a clear view of the fundus by the camera during the recordings.

After the patients’ pupils were fully dilated with 0.5% tropicamide and 0.5% phenylephrine hydrochloride, fmERGs were elicited by 5 Hz rectangular stimuli (100-ms light on and 100-ms light off). A total of 512 responses were averaged by a signal processor. A time constant of 0.03 seconds with a 100-Hz high-cut filter on the amplifier was used to record the a- and b-waves.

The amplitude of the a-wave was measured from the baseline to the first negative trough, and the amplitude of b-wave was measured from the trough of the a-wave to the positive peak of the b-wave. The noise level of our recording system was <0.4 μV.

Macular Thickness Measured by OCT

The macular thickness was measured by OCT (Stratus OCT; Carl Zeiss Meditec, Dublin, CA) before, and 1 week, 1 month, and 3 months after PDT. Six radial scans of 6-mm length were made by using the Fast Macular Thickness Map (FMTM) protocol. Because the FMTM system often misreads the exact borders of the retina in the presence of a CNV, the thickness of the retina was determined by our new program (Ishikawa K et al. IOVS 2005;46:ARVO E-Abstract 1550). For this program, the user is able to set eight or more cursors above and below a selected area manually, and for this study, the upper cursors were set on the internal limiting membrane (ILM), and the lower cursors were set on the retinal pigment epithelium (RPE) side of the retina. Another set of cursors was set at the fovea of the OCT images. When a CNV extended above the RPE, the lower cursors were set on the retinal side of the CNV contour. The program then automatically calculated the average macular thickness within a 3-mm (diameter) circle centered at the fovea.

Indocyanine Green Angiography

ICGA was performed with a confocal scanning laser ophthalmoscope (Heidelberg Retina Angiography II; Heidelberg Engineering, Dossenheim, Germany) before and 3 months after PDT. All eyes were classified into two groups from the ICGA findings at 10 minutes: group A included eyes with indistinct hypofluorescence at the site of the PDT, and group B included eyes with a well-defined hypofluorescence border coinciding with the site of the PDT. Eyes in which the border of the hypofluorescent area could be clearly recognized for 360° were defined as having well-defined hypofluorescence borders. This classification was performed by two retinal specialists (MK, HN) who were masked to the clinical information of the patients.

Statistical Analyses

The visual acuity, macular thickness, amplitudes, and implicit times of fmERGs after PDT were compared with the corresponding values recorded before by the nonparametric Wilcoxon signed rank test. The Mann-Whitney U test was used to compare two groups, and the Pearson’s correlation coefficient and the Fisher r-to-z test for analysis of correlation. The data were analyzed with commercial software (Stat-

| Table 1. fmERGs before and 1 Week, 1 Month, and 3 Months after PDT | Normal Subjects (n = 112) |
|---|---|---|---|---|
| **Visual acuity (logMAR)** | **Pre-PDT** | **Post-1 W** | **Post-1 M** | **Post-3 M** |
| *P* | 0.71 ± 0.32 | 0.63 ± 0.35 | 0.63 ± 0.36 | 0.65 ± 0.42 |
| **Amplitude (μV)** | **a-wave** | **b-wave** | **a-wave** | **b-wave** |
| *P* | 0.52 ± 0.53 | 0.36 ± 0.45 | 0.45 ± 0.41 | 0.47 ± 0.48 | 2.10 ± 0.64 |
| | 0.0008 | 0.0226 | 0.33 | 0.3873 |
| **P** | 0.92 ± 0.91 | 1.46 ± 0.72 | 1.49 ± 0.58 | 1.79 ± 0.67 | 4.89 ± 0.94 |
| | 0.0006 | 0.0009 | 0.1255 |
| **Implicit time (ms)** | **a-wave** | **b-wave** | **a-wave** | **b-wave** |
| *P* | 25.8 ± 3.3 | 57.0 ± 10.2 | 0.12 | 0.59/2 |
| | 27.2 ± 4.5 | 56.8 ± 9.2 | 0.9158 | 0.2353 |
| | 25.8 ± 3.8 | 54.9 ± 9.5 | 55.3 ± 8.9 | 0.2449 |

Data are the mean ± SD.

* Wilcoxon signed-rank test (compared with before PDT). Significant differences are in bold.
RESULTS

Visual Acuity

The mean best corrected visual acuity (BCVA) in logMAR units was 0.71 ± 0.32 (mean ± SD; 20/103, Snellen equivalent) before PDT, and it improved to 0.63 ± 0.35 (20/85) 1 week after PDT. The BCVA then stabilized at 0.63 ± 0.36 (20/85) and 0.65 ± 0.42 (20/89) at 1 and 3 months, respectively, after PDT. The BCVA at 1 week and 1 month was significantly better than that before PDT (P < 0.01 at 1 week; P < 0.05 at 1 month, Wilcoxon signed-rank test, Table 1).

For this study, an increase of >0.2 logMAR was defined as an improvement, and a decrease of >0.2 logMAR was defined as a worsening. A change in the BCVA of < ±0.2 logMAR was classified as unchanged BCVA. At 1 week after PDT, 29 (78%) eyes were unchanged, 8 (22%) had an improvement, and none had a decrease. At 3 months after PDT, 23 (62%) were unchanged, 10 (27%) had an improvement, and 4 (11%) had a decrease.

Focal Macular Electroretinograms

The mean amplitude of the b-wave was reduced to 76% and 78% of the pre-PDT amplitude at 1 week and 1 month after PDT, respectively. The b-waves then recovered to 93% at 3 months after PDT (Table 1). The reductions in the b-wave amplitude at 1 week and 1 month after PDT were statistically significant (P < 0.001, Wilcoxon signed-rank test).

Similarly, the amplitude of the a-wave was reduced to 59% of the pre-PDT value, and then gradually increased to 87% and 90% of the pre-PDT level at 1 and 3 months after PDT, respectively (Table 1). The reduction in amplitude at 1 week after PDT was statistically significant (P = 0.02).

There were no significant changes in the implicit times of the a- and b-waves within 3 months after PDT (Table 1).

A comparison of the amplitudes of the a- and b-waves of the fmERGs before (abscissa) and 1 week after (ordinate) PDT for the 37 eyes is shown in Figure 1. The amplitude of the a-wave was decreased in 16 of 37 eyes at 1 week after PDT. In 12 of 37 eyes, the amplitude of the a-wave was nonrecordable before and 1 week after PDT. The b-wave amplitude was decreased in 23 of 37 eyes at 1 week after PDT.

Macular Thickness

The macular thicknesses within a 3-mm-diameter circle before and 1 week after PDT in all 37 eyes are plotted in Figure 2. The macular thickness tended to be reduced slightly after PDT, but this reduction was not statistically significant (i.e., before PDT, the thickness was 338.9 ± 68.0 μm; and 1 week after PDT, it was 322.1 ± 83.5 μm; P = 0.06, Wilcoxon signed-rank test).

Next, we investigated whether the reduced fmERG seen 1 week after PDT was related to the changes in macular thickness at 1 week after PDT. The relative changes in the b-wave amplitude at 1 week after PDT were not significantly correlated with the relative changes in the macular thickness at 1 week after PDT (r = 0.197, Pearson’s correlation coefficient, P = 0.2436, Fisher r to z).

ICGA Findings

Finally, we studied the ICGA findings in 37 eyes at 3 months after PDT. We found that 16 (43%) eyes showed an indistinct hypofluorescence at the site of the PDT. (Figure 1, A) Eyes with an indistinct hypofluorescence at the site of the PDT (group A); (B) eyes with a well-defined hypofluorescence border coinciding with the site of the PDT (group B). (A) Amplitude of the a-wave of fmERG before (abscissa) and 1 week after (ordinate) PDT. (B) Amplitude of the b-wave before (abscissa) and 1 week after (ordinate) PDT.
hypofluorescence at the site of the PDT (group A), and 21 (57%) eyes showed a well-defined hypofluorescence border coinciding with the site of the PDT (group B). Representative ICGA at 1 and 10 minutes after infusion and the fmERGs for groups A and B are shown in Figures 3A and 3B. The clinical characteristics of the two groups are summarized in Table 2.

We examined whether there was any difference in the relative amplitudes of the fmERGs after PDT between the two groups and found that the relative b-wave amplitude at 1 week after PDT to that before PDT was significantly larger in group A (1.14 ± 0.62) than that in group B (0.65 ± 0.29; \( P = 0.0015 \), Mann-Whitney U test, Fig. 4A). This difference was still significant at 1 month after PDT (\( P = 0.03 \), Fig. 4B), but was not significant at 3 months afterward (\( P = 0.13 \)).

We also investigated whether there was any difference in the macular thickness at 1 week after PDT between these two groups. There was no significant difference in the relative changes in the macular thickness at 1 week after PDT between groups A (0.92 ± 0.20) and B (1.00 ± 0.25, \( P = 0.09 \)).

**DISCUSSION**

Our results demonstrated that the amplitudes of the fmERGs were transiently reduced after PDT, even though the visual acuity improved after treatment. Thus, at 1 week after PDT, the amplitudes of the a- and b-waves were reduced to 69% and 76% of the pre-PDT values, respectively, but they recovered to the pre-PDT level within 3 months. These changes in the fmERGs after PDT were similar to the results of some previous studies using mERGs.\(^9,10,12\) For example, Lai et al.\(^12\) reported that the amplitudes of the mERGs were reduced to 84% to 87% within 2 weeks after PDT, then recovered to the pre-PDT level at 1 month. Therefore, our results support the idea that the macular
The exact mechanism of this transient reduction of the macular ERGs after PDT has not been determined and is still controversial.\textsuperscript{9,10,12,13} We had hypothesized that the transient reduction of fmERG might be related to morphologic changes at early stages after PDT, because it has been reported that vascular leakage and/or subretinal fluid were transiently increased at the early stages after PDT in clinical\textsuperscript{18–20} and animal studies.\textsuperscript{21–24} To examine whether these morphologic changes in the macula were related to the reduction of the amplitude of fmERG, we measured the macular thickness (distance from the ILM to the RPE, including subretinal fluid) using the Stratus OCT (Carl Zeiss Meditec, GmbH) and our program (Ishikawa K et al. IOVS 2005;46:ARVO E-Abstract 1550). However, we could not find any significant correlation between the changes in the macular thickness and reduction of the fmERGs.

We next hypothesized that the transient amplitude reduction of fmERGs is related to the decrease in choroidal circulation after PDT and examined the ICGA findings. We found that the amplitude reduction of fmERG after PDT was more severe in patients who had a well-defined choroidal hypofluorescence at the site of PDT than in patients with indistinct hypofluorescence of ICGA at 1 week after PDT (Fig. 4A). This difference remained significant at 1 month after PDT (Fig. 4B). These results suggest that the transient amplitude reduction of fmERG after PDT is related to a dysfunction of the outer retina due to the reduced choroidal circulation caused by the PDT.

It is widely accepted that PDT is not perfectly selective for CNV, but can cause partial occlusion of the choroidal vessels. Choroidal hypoperfusion coinciding with the PDT site has been reported in clinical studies.\textsuperscript{18,19,26–29} In addition, animal studies have demonstrated that PDT can cause the occlusion of normal choriocapillaris, even though the occlusion is reversible.\textsuperscript{21–24} Recently, Tzekov et al.\textsuperscript{25} recorded mfERGs and ICGA in two cynomolgus monkeys, and reported that the amplitudes of the mfERGs were reduced to approximately 20% of the pre-PDT level within 1 week after PDT. They suggested that this reduction is due to reduced oxygen supply from the choroid to the outer retina caused by extensive closure of the choroidal vessels after PDT. Our clinical study supports this suggestion.

We also noted that after PDT, the amplitude reduction of the b-wave was more prolonged than that of the a-wave (Table 1). We could not determine the exact reason for this prolonged depression of the b-wave. One possibility is that the middle retinal layer, which is the origin of the b-wave, is also impaired after PDT, and that the functional recovery of middle retinal layer is more delayed than that of the outer retina. We have experienced similar phenomenon in patients with central serous chorioretinopathy.\textsuperscript{17}

There were some limitations to our study. First, we recorded ICGA before PDT and 3 months after PDT and did not examine the changes in the choroidal circulation at the early stages after PDT. Therefore, we could not determine whether a correlation exists between the fmERGs and ICGA findings at 1 week and 1 month after PDT. Second, we did not examine the morphologic findings by OCT at the very early stages after PDT (e.g., within 2 to 3 days after PDT). There is recent evidence that acute inflammatory changes after PDT are seen most clearly within 2 to 3 days after PDT.\textsuperscript{18–20} Such very early morphologic changes may be related to our fmERG findings. Third, the interpretation for the changes in the macular thickness after PDT is very complex. On the one hand, if there is retinal edema before PDT and if PDT is successful, then the macular thickness may be reduced after PDT, and this thinning may be associated with visual improvement. On the other hand, if PDT damages choroidal blood flow profoundly, then post-PDT macular thinning might be associated with impaired vision and ERG changes.

Finally, we could not determine which factors of the patients contributed to the relatively severe reduction of choroidal circulation after PDT. As shown in Table 2, patients who were older, had larger lesions, and were female tended to have a relatively greater reduction of choroidal circulation after PDT.

**Table 2. Data for Study Groups before PDT**

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes (n)</td>
<td>16 eyes</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
</tr>
<tr>
<td>Age (y)</td>
<td>67.2 ± 8.7</td>
</tr>
<tr>
<td>Visual acuity (logMAR)</td>
<td>0.81 ± 0.30</td>
</tr>
<tr>
<td>GLD (μm)</td>
<td>2901 ± 1060</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD. Group A: eyes with no or indistinct hypofluorescence borders coinciding with the site of the PDT; group B: eyes with well-defined hypofluorescence borders coinciding with the site of the PDT.

**Figure 4.** (A) Box plots of the relative b-wave amplitudes at 1 week after PDT to that before PDT in groups A and B. (B) Box plots of the relative b-wave amplitudes at 1 month after PDT to that before PDT for the two groups. Line within the box indicates the median, the box limits are the 25th and 75th percentiles, the error bars are the 10th and 90th percentiles. There were significant differences between the two groups both 1 week and 1 month after PDT. (P < 0.05, Mann-Whitney U test).
(group B). However, we are hesitant to conclude that these factors are really involved, because the number of patients was small. Further studies are needed to clarify what factors in the patients are related to the most important effect on choroidal circulation after PDT treatment.

References


