Changes in Multifocal Electroretinograms Induced by Transpupillary Thermotherapy

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Objective: To determine retinal function after transpupillary thermotherapy (TTT) for subfoveal choroidal neovascularization using multifocal electroretinograms (mfERGs).

Methods: Multifocal electroretinograms were recorded before and after TTT (wavelength, 810 nm; diameter, 3 mm; duration, 60 seconds; power, 350 mW) in 9 eyes in 9 patients with subfoveal choroidal neovascularizations. The stimulus consisted of 7 hexagons; the central hexagon covered the laser-irradiated area and the surrounding 6 hexagons covered the nonirradiated area. Each recording was completed within 1 minute, and mfERGs were recorded periodically during the first 60 minutes after TTT and also at 24 hours and 1 week after TTT.

Results: The amplitude of mfERGs from irradiated areas was significantly reduced at 1 minute after TTT (P<.01) and then recovered soon. The peak time was prolonged at 15 minutes after TTT (P<.01), recovered to pre-TTT levels at 60 minutes, and then was prolonged again at 24 hours (P<.05) and 1 week (P<.05) after TTT. The mfERGs in nonirradiated areas were unchanged during the observational period.

Conclusions: We found amplitude reduction in central focal ERGs at 1 minute after TTT, transient peak-time delay at 15 minutes, and a delay at 24 hours. Early reduction is probably directly caused by an increase in temperature during TTT as previously reported in focal flicker ERGs. Peak-time delays at 15 minutes and 24 hours may be caused by other factors, such as increased intracellular calcium (Ca²⁺), the release of nitric oxide or heat shock proteins, vasodilation, or change in choroidal neovascularization. Our findings indicate that recording mfERGs may be a useful tool for evaluating TTT procedures.

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Transpupillary thermotherapy (TTT) was introduced by Reichel et al as a method to treat choroidal neovascularizations (CNVs). They recommended using a long-duration, large-spot, and low-intensity irradiation from an infrared diode laser for this treatment. They found a decrease of exudation in 94% of their patients, an improvement of visual acuity in 19%, stabilization in 56%, and a decline in 25%. Other investigators reported similar results. The laser power in TTT should be determined for fundus pigmentation to avoid overtreatment or undertreatment because melanin effectively absorbs infrared light. However, such information is not enough for adjusting laser power in each patient. To observe the increase in temperature during laser irradiation, liposomal-encapsulated dyes were tried in animals. In addition, recordings of focal flicker electroretinograms (ERGs) during TTT showed a decrease of the amplitude caused by the increase in temperature using specially designed ERG equipment. These studies suggest that both liposomal-encapsulated dyes and focal flicker ERG could be used as indicators for temperature increase in TTT. Previously, we also indicated that flicker ERG is very sensitive to temperature change and reported that the multifocal electroretinogram (mfERG) originates from postreceptor activities that are similar to those of flicker ERG. In the present study, we recorded mfERGs before and after TTT, a widely used technique for detecting changes in retinal function induced by TTT.

Methods

Subjects

We studied 9 eyes in 9 Japanese patients with brown eyes. All of the subjects had a subfoveal choroidal neovascularization, and the TTT procedures were performed with the same equipment and method. The mfERGs were recorded before and after TTT, and the responses were analyzed using multifocal electroretinogram analysis software (Multifocal Analyzer; COACOM, France). The mfERGs were recorded before and after TTT, and the responses were analyzed using multifocal electroretinogram analysis software (Multifocal Analyzer; COACOM, France).
veal CNV and exudative retinal detachment, and all gave informed consent after we explained the purpose and procedures to be performed. The procedures used in this study conformed to the tenets of the Declaration of Helsinki (Table).

We performed TTT with a single irradiation of 3.0-mm diameter, 350-mW power, and 60-second duration. The subfoveal CNV (occult type) had been diagnosed with fluorescein and indocyanine green angiography and an optical coherence tomography scanner (OCT II; Zeiss Humphrey Systems, Dublin, Calif). Subretinal fluid was detected by optical coherence tomography in all eyes. The corrected visual acuity, measured with a standard visual-acuity chart (CV-300, Tomei, Japan) with 14 lines of Landolt rings was 0.3 or less in all eyes.

**mERG SETTINGS**

We recorded mERGs with the VERIS system (Science version 4.0; Electro-Diagnostic Imaging, San Mateo, Calif). The details of our recording technique were reported previously.14,15 In this study, we used 7 hexagons as the stimuli, and the overall pattern covered the central 40° of the visual field (Figure 1A). The central hexagon fell on the area irradiated by the laser (mERG from the irradiated area), and the surrounding 6 hexagons covered the nonirradiated area (mERG from the nonirradiated area). Because each hexagon stimulated a larger area than the standard setup (eg, 61 or 102 hexagons), the amplitudes of the mERGs were larger than those recorded in standard mERG recordings. In addition, the m-sequence exponent was reduced from $2^{15}$ (standard setting) to $2^{14}$, which shortened the recording time to 60 seconds (standard recording time is about 7 minutes). The reduction was found in all of the subjects. The amplitude at 1 minute after TTT was significantly smaller than the pre-TTT amplitude (Figure 1B).

We measured the amplitude and peak time of the first positive wavelet (Figure 1C).

**TTT AND mERG RECORDING PROCEDURES**

W performed the TTT and mERG recordings under ordinary room light with the pupil maximally dilated. We set up a slit-lamp combined with laser for TTT (OcuLight SLx; Iridex Corporation, Mountain View, Calif) next to the VERIS system so that we could record mERGs immediately after performing the TTT.

The control mERGs were recorded with a GoldLens (Diagnosys LLC, Littleton, Mass) before the TTT. Then, TTT was performed with a single irradiation (diameter, 3.0 mm; power, 350 mW; duration, 60 seconds) of an 810-nm infrared diode laser using a Goldmann 3-mirror lens. Immediately after TTT, the patient had mERG stimulus again, the Goldmann lens was replaced with the GoldLens, and mERGs were recorded periodically for 60 minutes. Because some of the patients had poor visual acuity, we held practice sessions before the actual recording to train the patients to fixate on the center of the monitor. This was especially important for the patient in case 6 who had a visual acuity of 0.01. In addition, we monitored the fixation with a fixation monitor during recording.14,15

We performed mERG recordings at later times (Table), but because mERG recordings can cause some discomfort, these recordings were made on a voluntary basis. As a result, the number of recordings was different for different patients.

We used paired $t$ tests on the changes in the mERGs before and after TTT to determine whether any changes were significant. A $P$ value of .05 was considered statistically significant.

**RESULTS**

We successfully recorded mERGs before and after TTT in all cases. The amplitude of mERGs from irradiated areas was significantly smaller at 1 minute after TTT, and the reduction was found in all of the subjects. The amplitude was not significantly smaller than the pre-TTT amplitude at 15 minutes (Figure 2A and B).

The peak time of mERGs from irradiated areas was prolonged at 15 minutes after TTT and recovered to pretTT levels at 60 minutes (Figure 2C and D). However, the peak time was again significantly delayed at 24 hours and 1 week after TTT (Figure 2C and D). Thus, significant delay was observed at 15 minutes, 24 hours, and 1 week. We found a delay of more than 1.66 milliseconds (2 sampling intervals) in 7 of 9 eyes. The peak time recovered to pretTT levels at 4 weeks after TTT (Figure 2D). Both the amplitude and peak time of ERGs on nonirradiated areas were unchanged at all times (data not shown).

The early changes in mERGs that occurred within 60 minutes after TTT are shown for a representative case.

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**Table. Patient Data**

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Eye</th>
<th>Pre-TTT Amplitude, µV</th>
<th>Latency, ms</th>
<th>Visual Acuity*</th>
<th>Approximate ERG Points After TTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/59</td>
<td>OS</td>
<td>0.64</td>
<td>29.1</td>
<td>0.3</td>
<td>24 h, 1 wk, 2 wk, 3 wk, 4 wk, 5 wk</td>
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<tr>
<td>2/M/66</td>
<td>OS</td>
<td>0.94</td>
<td>30.8</td>
<td>0.1</td>
<td>24 h, 1 wk, 2 wk</td>
</tr>
<tr>
<td>3/M/50</td>
<td>OD</td>
<td>1.16</td>
<td>29.1</td>
<td>0.4</td>
<td>24 h, 1 wk</td>
</tr>
<tr>
<td>4/M/51</td>
<td>OD</td>
<td>2.19</td>
<td>30.0</td>
<td>0.4</td>
<td>24 h, 1 wk</td>
</tr>
<tr>
<td>5/M/52</td>
<td>OD</td>
<td>0.96</td>
<td>33.2</td>
<td>0.2</td>
<td>24 h, 1 wk, 4 wk</td>
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<tr>
<td>6/M/56</td>
<td>OS</td>
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<td>25.0</td>
<td>0.01</td>
<td>24 h, 72 h, 1 wk, 2 wk, 3 wk, 4 wk, 5 wk</td>
</tr>
<tr>
<td>7/F/30</td>
<td>OD</td>
<td>0.64</td>
<td>25.8</td>
<td>0.2</td>
<td>24 h, 1 wk, 2 wk, 3 wk, 4 wk</td>
</tr>
<tr>
<td>8/M/69</td>
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<td>26.6</td>
<td>0.5</td>
<td>24 h, 1 wk, 2 wk, 3 wk, 4 wk</td>
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<tr>
<td>9/F/41</td>
<td>OS</td>
<td>0.68</td>
<td>29.9</td>
<td>0.2</td>
<td>24 h, 1 wk, 2 wk, 3 wk, 4 wk</td>
</tr>
</tbody>
</table>

*Visual acuity was measured with a standard chart (CV-300, Tomei, Japan) with 14 lines of Landolt rings.
(case 3) in Figure 3. Figure 3A shows the mfERG on the irradiated area and mfERGs from the nonirradiated area, and Figure 3B shows superimposed mfERGs on the irradiated area. At 1 minute after TTT, the amplitude of the ERG on the irradiated area was reduced, but the peak time was unchanged (Figure 3B). At 5, 15, and 20 minutes after TTT, the amplitude was almost equal to pre-TTT levels, but the peak time was prolonged (Figure 3A). At 1 hour after TTT, both peak time and amplitude were identical to pre-TTT levels. On the other hand, mfERGs on the nonirradiated area were unchanged. This was observed in 7 of 9 cases.
Figure 3. Multifocal electroretinograms (mfERGs) recorded during the 60 minutes after transpupillary thermotherapy (TTT) in case 3. A, The mfERG from the irradiated area (left) and mfERGs from a nonirradiated area (right). B, The superimposition of the mfERGs from the irradiated area.
**Figure 4** shows mfERGs after TTT during the entire observation period in another representative case (case 1). The early changes in the mfERG from the irradiated area were identical to those in case 3. At 24 hours and 1 week after TTT, the peak time was prolonged again, although the amplitude was unchanged. The amplitude and peak time of ERGs on the nonirradiated area were unchanged. The late changes in peak time after 24 hours were observed in the 7 cases that had the early change in the amplitude.

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**COMMENT**

We found 3 types of change in mfERGs after TTT: early reduction of the amplitude, early and transient delays of peak time, and later delay of the peak time. These changes were observed only in mfERGs from irradiated areas; mfERGs from nonirradiated areas were unchanged. These findings indicate that these ERG changes were induced by irradiation and not by other factors, such as intraocular pressure alterations from the use of the Goldmann contact lens or illuminations of the slitlamp.

It is most likely that these changes were related to the increase in temperature induced by laser. However, the exact time course of the changes in mfERGs from irradiated areas could not be determined because we were not able to record mfERGs continuously for a long time in human subjects. It is thus possible that the real changes in ERGs on irradiated areas after TTT are more complicated than we observed. To resolve this point, experiments on animals may be required. Further, we must note that the retinas over CNVs may not be normal, so these results may not entirely reflect normal responses to heat.

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**EARLY REDUCTION OF THE AMPLITUDE**

Falsini et al\(^{10}\) reported that a reduction of the amplitude without a change in the peak time occurred in flicker ERGs during TTT in human subjects. Although the temperature of the retina returns to normal immediately after TTT, this change in flicker ERGs requires about 1 minute to recover.\(^{10}\) The origins of flicker ERG have been studied intensively using dl-2-amino-4-phosphonobutyric acid (APB) and cis-2,3-piperidinedicarboxylic acid (PDA), which block on- and off-bipolar activity, respectively.\(^{13,16,17}\) The results of these studies suggest that the flicker ERG mainly originated from postphotoreceptor activity. The origins of mfERGs have also been studied using the same glutamate analogues and inhibitory neurotransmitters.\(^{12,18}\) The results of these studies indicated that activities of on- and off-bipolar cells contribute to mfERG. Thus, flicker ERGs and mfERGs originate from postphotoreceptor neurons located in same region in the retina.

Therefore, it is likely that early reduction of the amplitude in mfERGs was induced by the direct effect of an increase in temperature, as suggested by Falsini et al\(^{10}\) for flicker ERG.

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**EARLY AND TRANSIENT DELAYS OF PEAK TIME AND LATE DELAYS OF PEAK TIME**

The peak time of mfERGs from irradiated areas was not changed at 1 minute after TTT, but it was prolonged at 10 to 15 minutes and returned to pre-TTT levels at 60 minutes after TTT. This transient change may not be caused by a direct effect of temperature change because ERG changes caused by the temperature increase occurred more quickly.\(^9\)

Many investigations have been performed on the effect of increase in temperature (heat shock) on the tissues,\(^{19-23}\) and Desmettre et al\(^{23}\) reported that heat shock protein plays an important role in TTT. Other studies have shown that nitric oxide induces heat shock protein.\(^{24-26}\) Accumulation of heat shock protein reaches its maximum level 24 hours after heat shock, and that of nitric oxide reaches its maximum level 1 hour after heat shock.\(^{24}\) An increase of intracellular calcium (Ca\(^{2+}\)) and free radicals precedes nitric oxide generation.\(^{24,27}\) If some of these chemical products affect mfERGs, it may explain our results.

Abnormal implicit time without a reduction of the amplitude in mfERGs has been observed in the very early stage of diabetic retinopathy.\(^{28}\) It is possible that mild circulatory disturbance or slight leakage from the vessels delays the implicit time of mfERGs without affecting the amplitude. Culla et al\(^{29}\) observed decreased blood flow in retinal circulation without alteration to choroidal blood flow at 24 hours after TTT. They also found decreased choroidal blood flow 4 weeks or later after TTT. The retinal circulation change at 24 hours may contribute to late delay in the peak time of mfERGs. Lanzetta et al\(^{30}\) reported increased leakage from CNVs within 60 minutes after TTT in 66.4% of their patients, and they found absent leakage at 1 week in 54.1% of their patients. The increased leakage within 24 hours might cause the early peak-time changes of mfERGs in our patients.

The mechanism of the delays in the mfERGs on irradiated retinas was not determined, but it is likely that some TTT-induced biochemical cascades or secondary responses of vessels to heat shock alter the function of the retina.

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**EVALUATION OF TTT PROCEDURES IN PATIENTS USING mfERGs**

The early reduction of the amplitude at 1 minute was observed in all of the cases, and the early, transient delay of peak time at 15 minutes was found in 7 of 9 cases. Interestingly, these 7 cases also showed late delay of peak time.

Thus, our patients can be divided into 2 groups by ERG findings: patients who had only amplitude changes (2 cases) and patients who had both amplitude and peak-time changes (7 cases). Unfortunately, we could not determine which group has a better prognosis because our cases in this study varied so much in terms of the size and type of CNV and the number of patients was so small. The change of peak time that we found in this study indicates either that the laser irradiation was efficacious or that the sensory retina was damaged, which should be determined in further study. In either case, however, it seems this new ERG technique has useful applications.
Figure 4. Four multifocal electroretinograms (mERGs) after transpupillary thermotherapy during the entire observational period for case 1. The left column shows the mERG from the irradiated area, and the right shows the mERGs from a nonirradiated area.
Multifocal electroretinograms are usually recorded with 61 or 103 hexagons, and this is the first report using only 7 hexagons. The reduction of the number of the hexagons resulted in an increase of the stimulated area. Therefore, the amplitude of each focal response became larger, and we were able to reduce the m-sequence exponent, resulting in a shorter recording time. Multifocal electroretinograms with lower numbers of hexagons and lower m-sequence exponents were suited for the present study because we attempted to determine the rapid alterations of the retina. Our results that the ERG changes were observed only in mfERGs from the irradiated areas indicate that our protocol was successful in recording focal ERGs from stimulated retinas.

In conclusion, our results showed that the amplitude of the mfERGs was reduced at 1 minute after laser irradiation while the implicit time was prolonged at 15 minutes, recovered, and was again delayed at 24 hours. The early reduction of the amplitude was probably caused by the direct effect of an increase in temperature, and the changes in peak time may be related to other factors, such as nitric oxide and heat shock proteins, vasodilatation, or alteration in CNV. Because the mfERG technique is widely available, our findings may be useful for evaluating TTT procedures in individual patients.

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