Short Wavelength Light-Induced Retinal Damage in Rats
Koji Masuda and Ikuo Watanabe

Department of Ophthalmology,
Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan

Purpose: To evaluate short wavelength light-induced retinal damage in rats.

Methods: Pigmented rats were exposed to 300–500 nm wavelength light for 30 seconds, 1, 3, 10, 20, 60, or 90 minutes. Electroretinogram a-, b-, and c-waves were recorded 48 hours after the exposure.

Results: While the a- and b-wave amplitudes were reduced significantly after exposures of 60 minutes and longer, the c-wave amplitude was reduced after only 3 minutes of exposure.

Conclusions: Rat retinal pigment epithelium is more sensitive to damage by exposure to 300–500 nm wavelength light than the neural retina.

Key Words: C-wave, electroretinogram, pigmented rat, retinal light damage, short wavelength light.

Introduction

There are two classes of retinal photochemical damage.1 In class I damage, the photoreceptor cell damage by long exposure to a relatively low light level has an action spectrum identical to the absorption spectrum of rhodopsin. In class II damage, the retinal pigment epithelium (RPE) damage caused by short exposure to intense white or short wavelength light has an action spectrum peak in the ultraviolet (UV) range. Moreover, it was found that 350 nm light damaged the photoreceptor cells,2 whereas 441 nm light damaged the RPE.3

In blue-light hazard, focal retinal damage due to narrow spectrum spotlight exposure has been analyzed histologically or ophthalmoscopically in monkeys,2–4 in rabbits,5,6 and rats.7,8 There are no reports evaluating the amplitude of the electroretinogram (ERG) c-wave as a function of blue-light hazard in rats. We studied the functional damage of the retina and RPE exposed to the broad-band short wavelength light in rats. We compared the amplitudes of the a-, b-, and c-waves under different exposure times to determine the threshold exposure time for inducing retinal and RPE damage. As a result, we concluded that there is a difference in the level of damage between the neural retina and the RPE in these exposure conditions in the rat.

Materials and Methods

Light Exposure Set-up and Animals

The 300–500-nm short wavelength light was supplied by a 500-W xenon arc lamp with an infrared heat-blocking filter (HA-30; Hoya, Tokyo) and a short wavelength filter (B-390, Hoya) (Figure 1). The light intensity at the level of the cornea was 40 mW/cm² (IL1700 Radiometer/Photometer; International Light, Newburyport, RI, USA).

DA pigmented male rats (SLC, Hamamatsu) were used at 9 weeks of age. They were reared in a regular 12-hour light–dark cycle with a room illumination of 300 lux, room temperature of 22–26°C, and water and food ad libitum. All experimental procedures adhered to the Hamamatsu University School of Medicine Guidelines for Animals in Research.

The animals were anesthetized with an intraperitoneal injection of a mixture of ketamine/xylazine 125:10 mg/kg and mounted on small animal holders. The pupils were dilated with drops of 1% atropine, 0.5% tropicamide, and 0.5% phenylephrine hydro-
chloride. One eye of each animal was exposed to the short wavelength light for 30 seconds, 1, 3, 10, 20, 60, or 90 minutes in Maxwellian view, the other eye serving as an unexposed control. Six animals were used in each exposure time group.

Electroretinographic Procedure

After a 48-hour recovery in the dark, the animals were anesthetized again with an intraperitoneal injection of a mixture of ketamine/xylazine 125:10 mg/kg and mounted on small animal holders. They were kept dark-adapted for 15 minutes in an electrically shielded, darkened room at a temperature of 33–35°C. Before ERG recordings, the pupils were dilated with drops of 0.5% tropicamide and 0.5% phenylephrine hydrochloride.

The ERG responses were recorded with a cotton wick differential electrode placed on the superior corneal limbus with an indifferent Ag/AgCl skin electrode attached to the bare skin between the eyes. The stimulus light was generated by a 500-W xenon arc lamp. A neutral density filter and optical wedge filter were inserted in the light path. The stimulus light intensity at the cornea was 13 mW/cm² (IL1700 Radiometer/Photometer; International Light) on the 0 log unit of the neutral density filter. A 1.0 log unit filter was used on the a-, b-, and c-wave recordings.

Alternating-current (AC) ERG. The duration of stimulus was 5 milliseconds, and the interstimulus interval was 50 seconds. The high cut-off filter and the time constant were set at 1 kHz and 0.3 seconds, respectively. Ten recordings were averaged (ATAC-150; Nihon Kohden, Tokyo), and were recorded using an X-Y recorder (Yokogawa, Tokyo). The amplitude of the a-wave was measured from the trough of the a-wave to the bottom of the b-wave.

Direct-current (DC) ERG. A DC amplifier (DAM50; World Precision Instruments, New Haven, CT, USA) was used. The duration of stimulus was 5 seconds, and the interstimulus interval was 3 minutes. The high cut-off filter was set at 3 kHz. Five recordings were averaged. The amplitude of the c-wave was measured from the baseline to the peak of the c-wave.

The amplitudes of the a-, b- and c-waves of the exposed and unexposed eyes were compared. The shortest exposure time in which the exposed group showed significantly ($P < .01$) smaller amplitudes than the unexposed group was defined as the threshold exposure time. The Student $t$-test was used to determine the significance of the difference.

Histologic Procedure

After ERG recordings, the animals were euthanized, and the eyes of the 3-minute and 60-minute exposure groups were enucleated. Each retina was examined with a light microscope.

Results

ERG

The mean and standard deviation of the amplitudes of the a-, b-, and c-waves for different exposure durations are shown in Figure 2. The amplitudes of the a- and b-waves tended to be smaller in the longer exposure groups, being significantly smaller in the exposed eyes than in the unexposed eyes for exposure times of 60 minutes and longer. On the other hand, the c-wave amplitude was significantly smaller in the exposed eyes than in the unexposed eyes for exposure times as short as 3 minutes (each group, $n = 6; P < .01$).

Morphological Findings

Irregular projections of the RPE toward the rod outer segment were observed in the posterior pole in the 3-minute exposure group and compared with the control eyes. In addition to these findings in the 3-minute group, vacuolization of the rod outer segment and disarrangement of the outer nuclear layer (ONL) and the rod outer segment (ROS) were found in the 60-minute group (Figure 3).

Discussion

Many investigators have studied retinal photochemical damage with the ophthalmoscope, light-
and electron-microscope, or ERG in monkeys,\textsuperscript{2–4} rabbits,\textsuperscript{5,6} and rats.\textsuperscript{7,8} Noell reported in 1966\textsuperscript{9} that low-level white or green light exposure caused photoreceptor damage, and Ham et al\textsuperscript{4} reported in 1976 that intense blue-light exposure caused RPE damage. Ham et al\textsuperscript{4} reported, moreover, when examining the damage by 350 nm light and 441 nm light, that 350 nm light damaged mainly the photoreceptor cells,\textsuperscript{2} whereas 441 nm light affected most prominently the RPE.\textsuperscript{3}

In most of the studies on the blue-light hazard, focal retinal damage with narrow spectrum spotlight exposure was analyzed histologically or ophthalmoscopically. Putting et al\textsuperscript{5} reported that blue light was more harmful to the RPE when quantified by vitreous fluorophotometry as the blood—retinal barrier dysfunction induced by 400–520 nm blue light and 510–740 nm yellow light in pigmented rabbits. Hoppler et al\textsuperscript{6} reported that there were no essential morphological differences in the RPE damage in pigmented and albino eyes in rabbits caused by 400–550 nm blue light.

There have been some reports evaluating the amplitude of the c-wave as a function of the light damage to the retina. Ohuchi et al\textsuperscript{10} and Skoog and Jarkman\textsuperscript{11} reported that the amplitude of the c-wave was markedly reduced in comparison with the amplitude of the a- and b-waves in albino rabbits exposed to intense white light. Nilsson et al\textsuperscript{12} reported that a blue light-absorbing filter protects the RPE from light damage in albino rabbits exposed to intense white light. Graves and Green\textsuperscript{13} reported that albino rats reared in cyclic light had no detectable c-waves.

There are no studies evaluating the c-wave amplitude as a function of retinal damage caused by short wavelength light in rats.

We used pigmented DA rats in the present study because (1) the lenses of rats are remarkably transparent to blue light and UVA radiation,\textsuperscript{14} (2) the rat is the only species for which two different action spectra of photochemical damage have been established,\textsuperscript{15} and (3) the c-wave was negative in white Wistar rats.\textsuperscript{15}

Because the c-wave in the corneal ERG is a complex response composed of a positive wave derived from the RPE and a negative wave derived from the neural retina, it cannot simply be considered that the reduction in amplitude of the c-wave reflects RPE damage. We tried to evaluate the damage to the neural retina and RPE by comparing the threshold exposure time in which the a-, b-, and c-wave amplitudes were reduced significantly in the light-exposed group compared with the unexposed. The c-wave amplitude was reduced after only 3-minute exposure, but the a- and b-wave amplitudes did not show a significant reduction until 60-minute exposure. The c-wave reduction after the short exposure times with no significant diminution of the a- and b-waves indicates a decrease of the RPE function without damage to the neural retina.

Morphologically, the RPE change, irregular projections toward the ROS, was already observed in the 3-minute group in spite of the mild change in the ROS and the ONL. These findings reflected the normal a- and b-waves and the diminished c-wave. On the other hand, in the 60-minute group, the ROS and the ONL changes were more severe in addition to
the RPE change, and the a-, b-, and c-waves were all reduced markedly.

We demonstrated the possibility that the RPE is more susceptible to damage by the 300–500 nm wavelength light than the neural retina under the same exposure condition. However, it is unclear whether the damage to the neural retina was caused primarily by short wavelength light exposure or was secondary to the RPE change.

No key chromophores of the retinal photochemical damage have been identified; however, rhodopsin in the photoreceptor cells,16,17 amino acids, flavins, and heme proteins,19 cytochrome c oxidase,20 lipofuscin,21 and opsins22 in the RPE cells are regarded as damaging chromophores. The susceptibility of the chromophore in the RPE to blue light and UVA radiation, as well as many other complicated factors, may contribute to the mechanism of the difference in short wavelength light-induced damage between the photoreceptors and the RPE.

Figure 3. Electoretinogram (ERG) and light microscopic findings. Top: Direct-current (DC) ERG for c-wave recording. Middle: Alternating-current (AC) ERG for a- and b-wave recordings. Bottom: Light microscopic findings of retina in posterior pole. INL: inner nuclear layer, ONL: outer nuclear layer, ROS: rod outer segment, RPE: retinal pigment epithelium. Bar = 20 μm. RPE projection toward ROS (↓) is visible in 3-minute group (middle) and 60-minute group (right). In addition to this finding, there are vacuolization of ROS (*) and disarrangement of ONL and ROS in 60-minute group.

References