Utility of colored-light pupil response in patients with age-related macular degeneration

Ken Asakawa,1 Hitoshi Ishikawa,1 Yoshiaki Ichibe,2 Kimiya Shimizu2

1 Department of Orthoptics and Visual Science, Kitasato University School of Allied Health Sciences
2 Department of Ophthalmology, Kitasato University School of Medicine

Purpose: To evaluate the pupil response to chromatic light stimulation in patients with age-related macular degeneration (AMD).

Methods: Twenty-one patients with asymmetric AMD, ranging in age from 60 to 83 years (mean age, 71.9 years), were enrolled in the present study. In the healthy eye, the fundus, visual acuity, and visual field were normal (controls). Each patient was examined by recording the pupil response to red (635 nm) and blue (470 nm) light stimulation at intensities of 100 cd/m2 for 10 seconds. The pupil response was evaluated from the following two parameters: "percent pupil constriction" and "latency of pupil constriction" as the time required to contract the pupil diameter by 1 mm.

Results: The blue light stimulation caused a greater and more sustained constriction of the pupil than did the red light stimulation in all patients. There was a substantial prolongation of the pupil constriction time after red light exposure in only AMD-affected eyes (P < 0.0001).

Conclusion: Our findings suggest that the prolonged pupil constriction latency to red light stimulation reflects the outer retinal damage. The "latency of pupil constriction" might have potential parameters for early diagnosis and differentiation of diseases of the retinal outer layers.

Key words: age-related macular degeneration, colored-light stimulus, pupil response, latency time, melanopsin-containing retinal ganglion cell

Introduction

Age-related macular degeneration (AMD) is a relatively common disease in which the damage to the outer retina causes progressive loss of sight, often leading to blindness. It is estimated that about 30% of adults over the age of 75 have some signs of AMD, and that approximately 10% of these patients demonstrate advanced- or late-stage damage. AMD is also a major cause of irreversible vision loss that affects a large number of Japanese elderly people. Therefore, we need a screening test to detect early-stage AMD to help prevent blindness in routine clinical practice examinations. Screening for AMD is usually initiated by detection of metamorphopsia using an Amsler chart; diagnosis is confirmed by optical coherence tomography, fluorescein fundus angiography, and/or indocyanine green angiography. Pupillary response can be used in screening for optic neuropathy but cannot differentiate AMD from other outer retinal diseases.

On the other hand, it has long been known that human photoreceptors are made up of three cones (S, L, and M cones) and rods, but a novel photoreceptor containing an intrinsically photosensitive pigment called melanopsin was found recently in the retinal ganglion cells. These melanopsin-containing intrinsically-photosensitive retinal ganglion cells (ipRGCs) make a contribution to circadian entrainment, melatonin regulation, and regulation of the pupil diameter changes to light (the "pupil light response"). The pupil response mediated by the ipRGCs has different sensitivity and color characteristics from that mediated by rod and cone cells. The ipRGC-mediated pupil response has a selective sensitivity to blue light of a wavelength of about 470 nm, reacts through depolarization to high-intensity light stimulus after a long latent period, and shows a sustained constriction even after light stimulation (the "post-illumination pupil response").

Therefore, our study aimed to examine the utility of AMD detection by pupillary response to different colored
light stimulation. The newly pupil parameters for early AMD detection are also presented.

**Materials and Methods**

**Subjects**
The study protocol was approved at the Kitasato University School of Medicine Institutional Ethics Committee. This study followed the tenets of the Declaration of Helsinki for research involving human subjects, and informed consent was obtained from all subjects prior to their participation in the study.

Twenty-one patients with asymmetric AMD were enrolled in this study. They ranged in age from 60 to 83 years (mean age: 71.8 ± 7.2 years). Among the patients, there were 9 eyes with occult AMD (non-classic type), 6 eyes with predominantly classic AMD, and 6 eyes with minimally classic AMD. The 21 contralateral healthy eyes were used as controls. Therefore, we confirmed the normal eye examination with best-corrected visual acuity of 20/20, no visual field abnormalities, and no history of past or present ocular disease. The exclusion criteria of the patients were severe cataracts (grade III to V in the Emery-Little classification) and those having taken drugs affecting the pupil, particularly, pilocarpine and atropine. Also, this study examined patients without any systemic or ophthalmic diseases likely to affect the chromatic pupil response (apart from AMD).

The greatest linear dimension (GLD) value was 3,308 ± 1,316 μm (range: 1,200 to 5,950 μm). The mean log MAR visual acuity of the AMD eyes was 0.5 ± 0.3 (range: 0.8 to 1.4).

**Pupil recording**
Iriscorder Dual (Hamamatsu Photonics K.K.; Hamamatsu) was used to measure the pupil response induced by red light (at 635 ± 5 nm) and blue light (at 470 ± 7 nm) color stimulus at a light intensity of 100 cd/m². According to prior reports, these wavelengths and intensities were intended to stimulate rod, cone, and melanopsin input preferentially to the pupil light reflex.

Based on previously reported studies, and on our own work, with varying stimulus durations, we found that a duration of 10 seconds at this light intensity was sufficient to obtain both the transient and sustained components of the pupil light reflex while keeping the total testing time to <60 seconds.

After 15 minutes of dark adaptation in a dimly lighted room, pupil light response for 10 seconds to a red stimulus with monocular light stimulation (normal eye → AMD eye) was measured, dark adaptation was repeated, and then pupil response to a blue stimulus with monocular

![A. Pupil response was obtained from the baseline diameter before stimulation and the minimum diameter during stimulation. B. Only the trajectory of pupil diameter changes during light stimulation is shown. The pupil constriction latency was recorded as the pupil constriction time required to contract the diameter by 1 mm. Normalized pupil diameter means the changes pupil diameter before light stimulation converted as zero.](image-url)
light stimulation (the normal eye → the AMD eye) was measured. Any data with artifacts, e.g., blinking and fixation instability, were excluded from analysis.

A typical pupillary trace is shown in Figure 1A. The percent pupil contraction was plotted as a function of time to visualize the dynamics of the pupil movement in response to the stimulus paradigm (Figure 1B). The tracings were visually inspected, and the transient and sustained pupil responses were determined for the stimulus intensity. In the present study, transient pupil response was defined as the maximal percent change from baseline pupil size during a time window of 1 second after light stimulus onset. Sustained pupil response was the amount the pupil remained contracted after 10 seconds of light stimulation. Also, the definition of normalized pupil diameter was the change in baseline pupil diameter before light stimulation converted to zero.

We evaluated two parameters: the baseline pupil diameter before light stimulation and the minimum pupil diameter during the first second after light stimulation.

1. Percent pupil constriction (%) = [(baseline pupil diameter - minimum pupil diameter) / baseline pupil diameter] × 100
2. Latency of pupil constriction (seconds): the time required to contract pupil diameter by 1 mm.

Statistical analysis
Results are presented as mean ± standard deviation (SD). Correlation between the two parameters and GLD values were evaluated by the Pearson’s product-moment correlation coefficient test. Comparisons of these dates by red- and blue-light stimulation were statistically analyzed using analysis of variance and the Scheffe test.

P values of < 0.05 were considered statistically significant. Statistical analyses were performed using commercially-available statistical software (SPSS, version 20.0; IBM Corporation, Armonk, NY, USA).

Results

Percent pupil constriction
The differences of AMD type, namely occult AMD, predominantly classic AMD, and minimally classic AMD were not seen. There was no correlation between the percent pupil constriction and GLD values (r = -0.23) in all the AMD eyes. Pupil miosis with red-light stimulation was lower when compared with the control eye (26.6 ± 8.7% vs. 37.7 ± 7.5%; P = 0.0005) (Figure 2A). With blue-light stimulation, the percent pupil constriction of all the AMD-affected eyes was 38.4 ± 8.4% compared with 46.8 ± 7.6% for the control eye (P = 0.02). The difference between responses to red and blue light for all the AMD eyes was statistically significant with a greater decrease in the eyes stimulated by red light (P = 0.0002).

Latency of pupil constriction
There were no differences among the AMD types. Latency of pupil constriction showed no correlation with GLD values (r = -0.22) in any AMD eyes. With the baseline pupil diameter before light stimulation used as zero, the latency of pupil constriction (time required to contract the pupil diameter by 1 mm) to red-light stimulation was considerably prolonged in eyes with AMD (AMD eye 0.73 ± 0.15 second vs. control 0.60 ± 0.09 second, P = 0.0071). A significant difference between the AMD eye and the control was not seen with

A. Percent pupil constriction with red-light stimulation was lower than with blue-light stimulation, and the AMD-affected eyes under red-light stimulation were significantly less constricted compared to the normal eyes and to the AMD-affected eyes under blue-light stimulation.

B. Latency of pupil constriction with red-light stimulation was considerably prolonged only in the AMD-affected eyes.
blue-light stimulation (AMD eye 0.54 ± 0.08 second vs. control 0.50 ± 0.07 second, P = 0.75) (Figure 2B). The difference between responses to red and blue light for the all AMD eyes was statistically significant, with a greater prolong in the eyes stimulated by red light (P < 0.0001).

Figure 3 shows an example of the waveform of a patient with AMD. The trajectory of the pupil diameter changes during light stimulation is shown. With red-light stimulation, prolongation of latency to pupil constriction was more clearly noted in the affected eyes, although no significant difference between the normal and affected eyes was found with blue-light stimulation.

**Discussion**

We examined the AMD detection in pupillary response related to different colored light stimulation, and the advantages of a newly pupil parameter for early-stage AMD detection were provided. Our results demonstrated that blue-light stimulation caused a greater and more sustained constriction of the pupil than did red-light stimulation. The most remarkable finding in our study was that the latency of pupil constriction to red light was prolonged in AMD but that this effect was not seen with blue-light stimulation.

Gamlin et al. evaluated sustained pupil constriction after blue-light stimulus and found that it was consistent with the spectral curve of melanopsin in humans. Several other investigators have also reported greater and sustained pupillary constriction after blue-light stimulation in humans. Generally, the blue-light stimulus activates mostly rods and S cones, in addition to any possible activation of ipRGCs. The ipRGCs, which drive depolarizing responses, have a longer latency time; this differs from cone cells, which respond instantaneously to light stimulation. It has been demonstrated that sustained pupil constriction is related to the slow response time of ipRGCs, so that the response to light stimuli achieves a maximum amplitude after a long latent period. Dacey et al. recorded action potentials of ipRGCs and showed that a blue-light stimulus produces significant cell spikes with a sustained response that slowly disappeared after the light stimulus was discontinued; for a red-light stimulus, the response attenuated instantly (adaptation). These results are also consistent with past reports derived from electrophysiological records of ipRGCs.

Young et al. newly mentioned that a transient pupil response immediately after red- or blue-light stimulus is mainly L- or M-cone cell-mediated response or slightly S-cone, ipRGC-mediated response, that a response during red- and blue-light stimulus is a cone-cell-mediated and ipRGC-mediated response, and that a pupil change after blue-light stimulus is discontinued is an ipRGC-mediated response.
In the present study, "latency of pupil constriction" was defined as the time required reaching a pupil diameter contraction of 1 mm. Although latency of pupil constriction is more likely to be influenced by the mechanical differences of each subject's iris, the latency time to the red-light stimulus was prolonged in the AMD-damaged eye, while no changes were noted between the control and AMD eyes in response to blue-light stimulation, strongly suggesting damage to the cone cells.

Furthermore, there was no correlation between either the percent pupil constriction or the latency of pupil constriction and the GLD values, which indicated the degree and size of degeneration. Considering this relationship between structural change in outer retina and functional change in two parameters, it may reflect that the change in the pupil response precedes GLD values abnormality in early stage AMD.

The limitation to the present clinical study findings pertains to the pupil response to blue-light stimulation and the possible contributory effects of the rod cells to this response. Further study of the rod cell contribution to pupil reactions to colored light stimulation should be pursued. In addition, further study for inherited macular dystrophy with progressive decrease of visual acuity but with essentially normal fundus and fluorescein angiograms such as occult macular dystrophy are warranted.

These results, in the present study, showed that the measurement of latency of pupil constriction in response to colored light could be used for the early diagnosis of diseases of the associated outer retinal layers.

Acknowledgments

This work was supported by JSPS KAKENHI, Grant No. 24791871 and a grant from Kitasato University School of Allied Health Sciences (Grant-in-Aid for a Research Project, 2013-1059).

References

