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Processes of Blue Light–Induced Damage to Retinal Pigment Epithelial Cells Lacking Phagosomes

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Purpose: To experimentally clarify the processes of the changes induced by blue light directly on the retinal pigment epithelium (RPE) before the formation of phagosomes or the accumulation of lipofuscin.

Methods: We developed a new experimental method in which primary cultured cells of very young pigmented rats were exposed to several intensities and durations of blue light (wavelength = 440 ± 10 nm).

Results: At 1.0 mW/cm^2, the damage was limited to mitochondria. At 2.0 mW/cm^2, the cytoplasm exhibited large whorls of membrane or whorled inclusions, which were consistent with autophagic vacuoles. At 4.0 mW/cm^2, the RPE cells showed lysis of the cytoplasm and a nucleus that was consistent with necrosis.

Conclusions: Our results suggested that damage induced by blue light to cultured RPE cells may originate in the mitochondria and end in necrosis. The type of cell death induced in the RPE by blue light seems to be determined mainly by the intensity of the light, but is also related to the duration of exposure. **Jpn J Ophthalmol 1999;43:103–108**

Key Words: Blue light damage, necrosis, pigmented rats, retinal pigment epithelium, ultrastructural damage.

Introduction

Radiant energy can affect the retina by three mechanisms: mechanical, thermal, and photochemical.[1] Photochemical reactions are initiated by ultraviolet or visible radiation that can induce changes in the absorbing tissue without elevating the temperature more than 10°C.[2–4] The retina,[3,5,6] including retinal pigment epithelium (RPE),[4,7–9] is much more sensitive to experimental photochemical damage from blue or green light than from longer wavelengths of visible light.

Damage caused by blue light has been implicated in phototoxicity to the neuroretina and/or RPE.[3,4,10–15] The accumulation of lipofuscin is one of the most characteristic features of aging observed in RPE cells.[16–18] Lipofuscin-loaded RPE cells were more sensitive to visible blue light than unloaded control cells.[19] The lipofuscin is believed to represent the chemically modified residuals of incompletely digested photoreceptor outer segments.[17] Whether this incomplete digestion is due to the peroxidized photoreceptor outer segments[19] or dysfunction of the RPE cells[20] has been unclear.

Recently, we have developed a primary culture of RPE cells that contain no outer segments[21] to investigate the direct damage to RPE by blue light.[22] We have proved that phagosome-free RPE cells can be damaged directly and show typical changes, such as large whorls of membrane or whorled inclusions, after intermittent exposure to blue light (440 nm ± 10 nm) for 36 hours at 2.0 mW/cm^2. However, our results could not reveal the processes of the RPE changes caused by light damage. In the present study, to clarify these processes, cultured RPE cells were directly exposed to different intensities and durations of blue light instead of a constant and fixed blue light, using the previously reported system.[22]

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Table 1. Retinal Pigment Epithelium (RPE) Changes After Exposure to Different Intensities and Durations of Blue Light

Culture No.	Exposure Duration (hour × day)	Exposure Irradiance (mW/cm^2)	Damage Rate of Exposed RPE Cells with Morphological Changes			
			Nuclear Lysis	Swelling of Mitochondria	Whorls of Membrane or Whorled Inclusion	Swelling, Vacuolation, or Disappearance of Organelles
1	12 × 3	1.0	0/61	2/61	0/61	0/61
2	12 × 3	1.0	0/67	2/67	0/67	0/67
3	4 × 3	2.0	0/93	15/93	1/93	0/93
4	4 × 3	2.0	0/35	6/35	1/35	0/35
5	12 × 3	2.0	0/39	16/39	6/39	0/39
6	12 × 3	2.0	0/126	33/126	9/126	0/126
7	12 × 3	2.0	0/53	25/53	15/53	0/53
8	12 × 3	4.0	25/43	40/43	1/43	38/43
9	12 × 3	4.0	27/34	30/34	1/34	30/34

Materials and Methods

Primary Culture of RPE and Light Exposure

Long-Evans rats were sacrificed 8–10 days after birth and the RPE cells were isolated and cultured in the method reported previously.[22] Treatment of the animals was consistent with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. One week after seeding, a monolayer culture of RPE was formed and used in our experiments. Exposure of the cells was performed as in our previous study.[22] The diameter of the exposed area was 1–3 cm. The samples were taken only from the central part of the exposed area. In each time period, one dish of RPE cells was exposed to blue light ($\lambda = 440 \pm 10$ nm) for a certain duration and intensity (See Table 1), whereas another dish served as the control. Control cells were placed in the same incubator and covered with aluminum foil. The constancy of temperature in the exposed area was monitored by an electronic thermometer (PC-2200; Delta, Tokyo) with an accuracy of ±1.0°C. Incubation was continued for 12 hours after exposure. A diagram of the equipment for blue light exposure is shown in Figure 1.

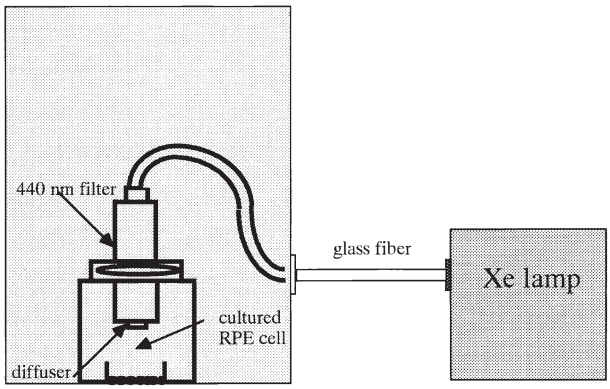

Figure 1. Diagram of equipment for blue light exposure.

Electron Microscopic Observations

After discarding the growth medium and washing the cell surface with phosphate-buffered saline (PBS), 2.5% glutaraldehyde was used to fix the exposed and unexposed control RPE cells in the dishes. Following overnight fixation, 1% osmium was used for postfixation, a series of ethanol solutions from 50% to 100% were used for dehydration, Epon mixture was then used for embedding, and ultrathin sections were made after staining. Finally, morphological findings were studied by transmission electron microscope (H-600; Hitachi, Tokyo).

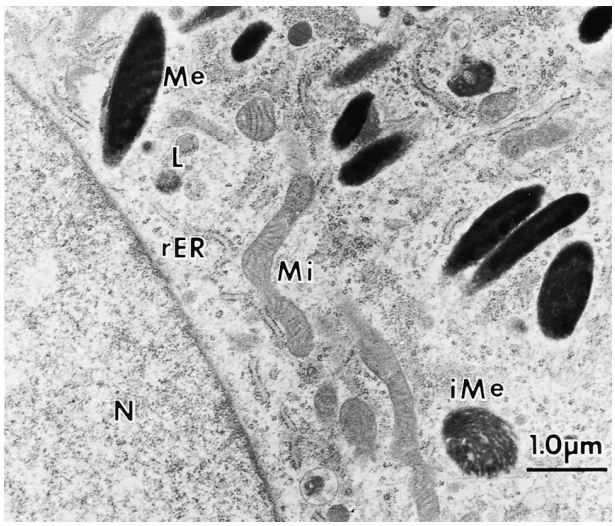

Figure 2. Cultured retinal pigment epithelium (RPE) cells (control): typical ultrastructure of cultured RPE in young pigmented rat. N: nucleus, Mi: mitochondrion, rER: rough endoplasmic reticulum, L: lysosome, Me: melanin granule, iMe: immature melanosome.

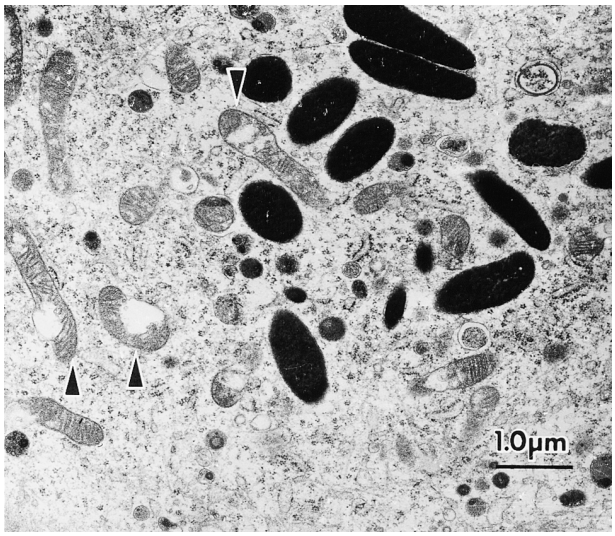

Figure 3. Cultured retinal pigment epithelium cells exposed to 36 hours blue light at 1.0 mW/cm^2. Mitochondria showed swollen state. Some had become disrupted (arrowheads).

Results

The morphology of the primary cultured RPE cells of the control was similar to that described in our previous report.[22] Electron microscopic observations showed that these cells contained a large number of melanin granules, lysosomes, well-developed rough endoplasmic reticulum (rER), and mitochondria (Figure 2). In the primary cultured RPE cells exposed to 12–36 hours of blue light at 1.0–4.0 mW/cm^2, the use of transmission electronic microscopy demonstrated a series of ultrastructural changes. The RPE damage after exposure to blue light at 1 mW/cm^2 for 36 hours was limited to mitochondria, which revealed swollen states with disrupted membranes (Figure 3). The whorls of membrane or whorled inclusion appeared after exposure to blue light for 12 hours at 2.0 mW/cm^2. The structures, arranged in membranous loops, were called whorls of membrane (Figure 4). Sometimes small whorls that seemed to originate from mitochondria could also be observed (Figure 4). Whorls of membrane were easily observed within an inclusion that was called whorled inclusion (Figure 5). The debris of the cytoplasm could also be found within the inclusion that was consistent with the autophagic vacuoles (Figure 5). The membranous loops became larger after exposure to blue light for 36 hours at 2.0 mW/cm^2. They were called large whorls of membrane (Figure 6), as described by us previously.[22] In those cells where the large whorls of membrane concentrated, dilated eER could also be detected (Figure 6). No marked decrease in the number of pigment granules was observed, and there was no apparent change in the nucleus after exposure to blue light for 12–36 hours at 2.0 mW/cm^2. In the primary cultured RPE cells exposed to 36 hours of blue light at 4.0 mW/cm^2, the cells showed lysis of the nucleus and cytoplasm (Figure 7). The karyoplasm showed granulation of the chromosomes. Most of the nuclear membrane had disappeared. The normal structure of the organelles in the cytoplasm was difficult to detect except for the melanin granules. The morphological changes in RPE cells induced by different intensities and durations of the blue light and the percentage of the cells in which specific changes appeared are shown in Table 1.

Discussion

It is believed that blue light around 440 nm readily damages the RPE.[4,10,13] With this model, we demonstrated the different types of RPE damage after exposure to blue light of 1.0–4.0 mW/cm^2. As described in our previous report, large whorls of membrane or

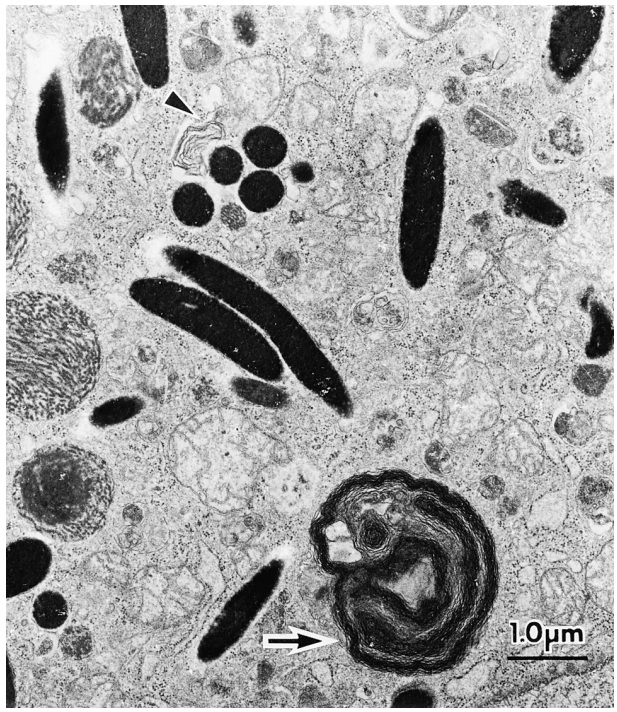

Figure 4. Cultured retinal pigment epithelium (RPE) cells exposed to 12 hours blue light at 2.0 mW/cm^2. Cytoplasm of degenerated RPE cell showed whorls of membrane (arrow). Minor whorls of membrane were forming from damaged mitochondria (arrowhead). No obvious changes were observed in nucleus or melanin granule.

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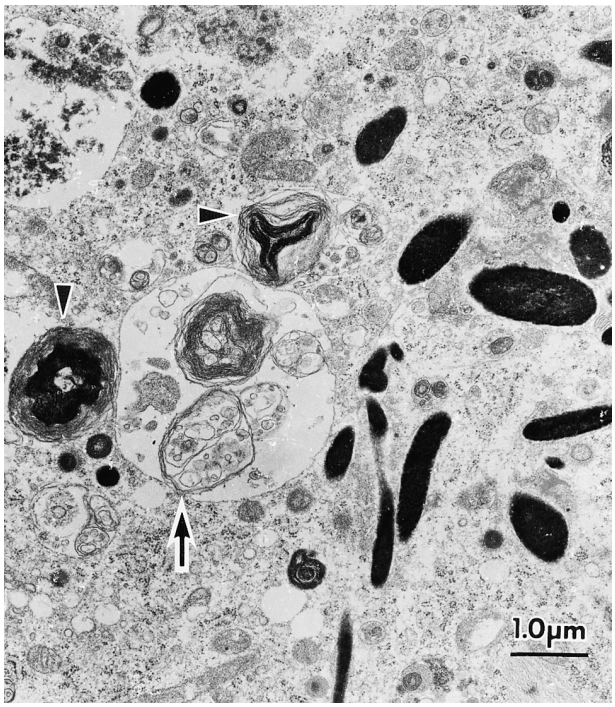

Figure 5. Cultured retinal pigment epithelium (RPE) cells exposed to blue light for 12 hours at 2.0 mW/cm^2. Cytoplasm of degenerated RPE cell showed whorls of membrane (arrowheads). Whorls of membrane and debris of cytoplasm could also be observed within whorled inclusion (arrow).

whorled inclusions appeared in cytoplasm of the RPE cells after exposure to blue light at 2.0 mW/cm^2 for 36 hours.[22] In the present study, we found the morphological change induced by blue light at 1.0 mW/cm^2 for 36 hours was limited to the level of mitochondria, as reported by Paulter and associates.[12] The whorls of membrane or whorled inclusions began to appear and occupied only a small part of the cytoplasm after exposure to blue light for 12 hours at 2.0 mW/cm^2. The whorls of membrane became larger and occupied more space in the cytoplasm after exposure to blue light for 36 hours at 2.0 mW/cm^2. Although there was no direct evidence to show the true origin of the large whorls of membrane that appeared in RPE cells after exposure to blue light at 2.0 mW/cm^2, some small whorls had been observed to originate from the damaged mitochondria in RPE cells after exposure to blue light for 12 hours at 2.0 mW/cm^2. Similar structures with large whorls of membrane were also accumulated in the RPE and other cells induced by sodium iodate and gentamicin toxicity.[23–29] The accumulation was possibly due to a lowered activity of some lysosomal enzymes, ie, sphingomyelinase and phospholipase.[23–26]

Extensive injury to cells by chemicals and other noxious stimuli often lead to necrosis.[30,31] The typical changes of lysis in the nucleus and cytoplasm of RPE cells after exposure to blue light at 4.0 mW/cm^2 for 36 hours proved that the necrosis of cultured RPE could be induced by a higher intensity of blue light. In fact, necrosis also appeared in the RPE cells following gentamicin injection with higher doses than could induce the accumulation of lipid inclusions.[28]

Autophagy, which is the sequestration of intracellular components and their subsequent degradation into secondary lysosomes, occurs even in normal cells, where it is related to cytoplasmic turnover. The phenomenon is greatly increased in cells that have been pathologically treated in various ways.[32] At the ultrastructural level, the main criterion for recognizing autophagy is the presence of cytoplasmic components with vacuoles. After exposure to blue light at 2.0 mW/cm^2, the whorled inclusions in which the cytoplasmic components could still be recognized, had a structure similar to the autophagic vacuoles. The inclusion shown in Figure 5 might represent an au-

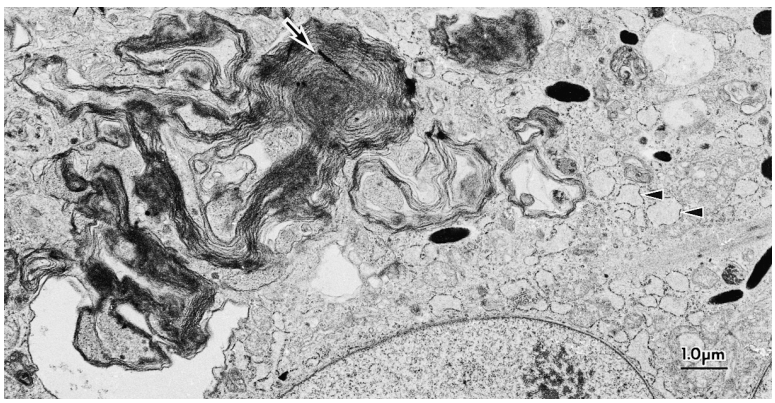

Figure 6. Cultured retinal pigment epithelial (RPE) cells exposed to blue light for 36 hours at 2.0 mW/cm^2. Cytoplasm of degenerated RPE cell showed large whorls of membrane (arrow). Dilated rough endoplasmic reticulum could also be detected (arrowheads).

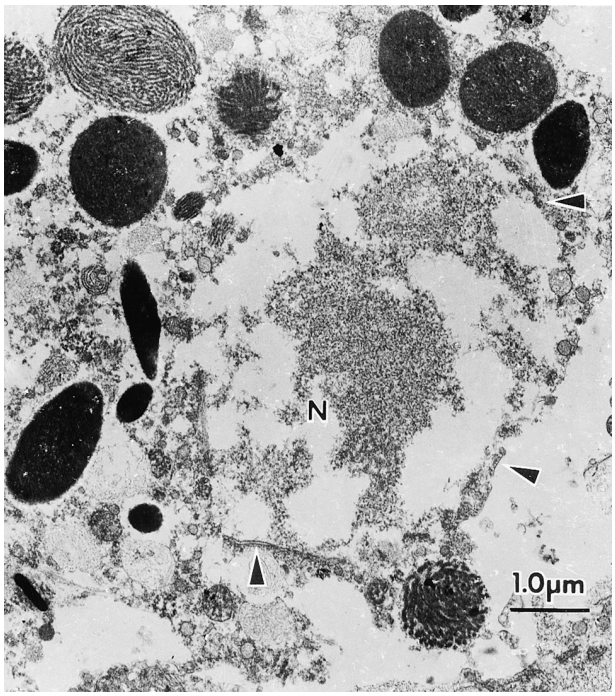

Figure 7. Cultured retinal pigment epithelium cells exposed to blue light for 36 hours at 4.0 mW/cm^2. In this cell, karyoplasm showed granulation of chromosomes. Although lysis of nuclear membrane was very obvious, some remnants of nuclear membrane could still be detected (arrowheads). Normal structure of organelles in cytoplasm was difficult to detect except for melanin granules. N: nuclear remnants.

tophagosome and an autolysosome. Many researchers believed that the autophagic vacuole is the result of a defensive reaction of the cell to isolate the altered region by various stimuli, although it is somehow linked to degeneration.[33,34] They did not agree that autophagy was instrumental in bringing about cell death. This seems reasonable because there is no evidence that the nucleus is ever the object of degradation within an autophagosome of a living cell.[35] On the other hand, the presence of numerous autophagic vacuoles in dying cells has also been reported.[36]

Clark[36] insisted that the irreversible destruction of the cytoplasm by the autophagic vacuoles will also result in cell death (type II cell death), especially when the total area of the autophagic vacuoles and dense bodies is roughly equal to, or greater than, that of the cytosol and organelles outside the vacuoles. This was in agreement with our results. In our results, the occupation of space by the large whorls of membrane and whorled inclusions or the dilatation of rER and swelling of mitochondria in some cytoplasms of the RPE cells after light exposure at 2.0 mW/cm^2 for 36 hours should indicate the increase in permeability. These might mean that autophagic cell death had happened (or was happening) in those cells. The whorls of membrane and whorled inclusions could be induced in the cytoplasm of the RPE cells after exposure for 12 hours at 2.0 mW/cm^2. Those structures were smaller in size and the number of the involved cytosol and organelles was also fewer. They occupied only a small space in the cytoplasm of the involved RPE cells. Only autophagic degeneration instead of cell death might have occurred.

From this study, we know that the type of damage was mainly determined by the intensity of the exposed blue light. However, longer duration (36 hours) of exposure to RPE cells at 2.0 mW/cm^2 of blue light might lead to autophagic cell death, whereas shorter duration (12 hours) would not lead to cell death. Different explanations have been given for blue light toxicity: (1) damage to blue-sensitive chromophores;[37] (2) damage to RPE, possibly associated with melanin;[3,4] and (3) damage due to the direct action of blue light by photosensitized aerobic oxidation, which could involve not only RPE and photoreceptors, but also cells in the other layers of the retina.[10] The hypothesis of photosensitized aerobic oxidation seems consistent with our results. We did not find any relationship between the melanin granules and the blue light damage.[22]

Our study suggests RPE cell death induced by blue light was mainly determined by the intensity of exposure, but is also related to duration of exposure. Further work should be done to elucidate the mechanism of the retinal damage produced by blue light exposure.

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