Potential Protective Role of TRPM7 and Involvement of PKC/ERK Pathway in Blue Light–Induced Apoptosis in Retinal Pigment Epithelium Cells in Vitro

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Purpose: Blue light triggers apoptosis of retinal pigment epithelium (RPE) cells and causes retinal damage. The aim of this study was to elucidate the protective role of transient receptor potential melastatin 7 (TRPM7) in photodamaged RPE cells.

Methods: RPE cells were isolated from Sprague-Dawley (SD) rats and exposed to varying intensities of blue light (500–5000 lux) in vitro. Cell proliferation and metabolic activity were respectively assessed by bromodeoxyuridine (BrdU) incorporation and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assays. Real-time polymerase chain reaction (RT-PCR) and western blotting were used to analyze the TRPM7, protein kinase C (PKC), extracellular signal-regulated kinase (ERK) and Bcl2-associated x/B-cell lymphoma 2 (Bax/Bcl-2) messenger RNA (mRNA) and protein expression levels. The cells were transfected with TRPM7 small interfering RNA (siRNA) or transduced with TRPM7-overexpressing lentiviruses and cultured with or without the pigment epithelium-derived factor (PEDF).

Results: Blue light inhibited the proliferation and metabolic activity of RPE cells in an intensity-dependent manner when compared to nonirradiated controls (P < 0.05). Compared to the control, photodamaged RPE cells showed decreased levels of TRPM7, PKC, ERK, and Bax, and an increase in Bcl-2 levels (P < 0.01). Forced expression of TRPM7 partially rescued the proliferative capacity of RPE cells (P < 0.01) and restored the levels of TRPM7, PKC, ERK, and Bax (P < 0.01), whereas TRPM7 knockdown had the opposite effects (P < 0.01). TRPM7 and PEDF synergistically alleviated the damaging effects of blue light.

Conclusions: Blue light triggers apoptosis of RPE cells, and its deleterious effects can be partially attenuated by the synergistic action of TRPM7 and PEDF via the PKC/ERK signaling pathway.

Key Words: apoptosis, light-induced damage, retinal pigmented epithelium, signal pathway, transient receptor potential melastatin 7 (TRPM7)

(Asia Pac J Ophthalmol (Phila) 2021;10:572–578)
shown that TRPM7 plays a neuroprotective role in the ischemic brain injury and oxygen-glucose deprivation (OGD) model and alleviates oxidative stress and inflammation.\textsuperscript{24,35} However, the pathways underlying the neuroprotective, proliferative, and antioxidant effects of TRPM7 remain to be elucidated.

In this study, we established an in vitro model of blue light retinal damage by irradiating rodent RPE cells with blue light. Cell proliferation and metabolic activity were respectively assessed by bromodeoxyuridine (BrDU) incorporation and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assays. Real-time polymerase chain reaction (RT-PCR) and western blotting were used to analyze the TRPM7, PKC, ERK, and Bcl2-associated x/B-cell lymphoma 2 (Bax/Bcl-2) messenger RNA (mRNA) and protein expression levels. The cells were transfected with TRPM7 siRNA or transduced with TRPM7-overexpressing lentiviruses and cultured with or without the PEDF. Our findings provide new insights into the pathogenesis, prevention, and treatment of retinal photodamage.

**METHODS**

**Cell Culture and Blue Light Exposure**

RPE cells were isolated from Sprague-Dawley (SD) rats as per guidelines of the Association for Research in Vision and Ophthalmology (ARVO) Statement on the Use of Animals in Ophthalmic and Vision Research. Rat RPE cells (CHI Scientific, Inc.) were cultured in Dulbecco’s Modified Eagle Medium/Ham’s Nutrient Mixture F-12 (DMEM/F12) supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin (all from Gbico, US) in a humidified incubator at 37°C with 5% CO2. The medium was changed every 2 days, and the cells were harvested and replated 1:2 once they were 90% confluent. The cells from passages 2 to 4 were seeded in 6-well plate transwell chambers at the density of 4 × 10^5/mL and cultured for 18 h. The cells were then irradiated with varying intensities (0, 500, 1000, 2000, and 5000 lux) of blue light for 6 h (group A), or with 2000 lux of blue light for 6 h (group B), and then cultured for 24 h.

Nonirradiated controls were also included. The medical blue light lamp (THOTH, Nanjing, F20T8/45, a maximum irradiance wavelength of 450 nM and an output power of 4.0 mW/cm^2) was placed 2000, and 5000 lux) of blue light for 6 h (group A), or with 2000 lux of blue light for 6 h (group B), and then cultured for 24 h.

**SiRNA Transient Transfection**

TRPM7 specific small interfering RNA (siRNA) and scrambled RNA (control siRNA) were purchased from Sigma Biotechnology (Sigma, US). The siRNAs transfected into RPE cells using the RPE cells from passages 3 to 6 were seeded in 6-well plates at the density of 1 × 10^5 cells/well in DMEM/F12 supplemented with 10% FBS. Once the cells were 60% to 70% confluent, the medium was replaced with serum-free DMEM/F12 and cultured further for 24 h. The cells were then transfected with 20 nM siRNA using Lipofectamine RNAiMAX transfection reagent (Invitrogen, US) in serum-free OPTI-MEM (Invitrogen, US). After 6 h, the medium was replaced with serum-free DMEM/F12. The transfection effects of TRPM7 siRNA were evaluated by measuring the expression level of TRPM7 by quantitative PCR (q-PCR) and western blotting.

**TRPM7 Lentiviral Transduction**

Cells in the exponential growth phase were plated in 6-well plates at the density of 1 × 10^5 cells/well. Once the cells were 60% to 70% confluent, the medium was replaced with serum-free DMEM/F12, and the cells were cultured for 24 h and then incubated with the control or TRPM7 lentivirus (specific lentivirus, mRNA: NM_053705.2) for 24 h in serum-free OPTI-MEM. After 24 h, the transfection medium was replaced with serum-free DMEM/F12. The transfection effects of TRPM7 lentivirus were evaluated by measuring the expression level of TRPM7 by q-PCR and western blotting.

**Statistical Analysis**

The data were presented as mean ± standard error of mean (SEM) of 3 independent experiments. Statistical analysis was performed using the SPSS software (version 19.0). The different groups were compared by analysis of variance (ANOVA), and P < 0.05 was considered statistically significant.
RESULTS

Characterization of RPE Cells

As shown in Figure 1, more than 95% of the cultured primary RPE cells from the third passage were positive for the epithelial marker CK-18. In addition, the typical cells exhibited the typical hexagonal, polygonal or fusiform morphology, and were relatively uniform in shape and arranged in a “paved stone” manner.

Blue Light Exposure Decreased the Proliferation of RPE Cells

Exposure to blue light significantly decreased the metabolic activity of RPE cells in an intensity-dependent manner compared to the nonirradiated control ($P < 0.01$; Fig. 2A). Consistent with the results of MTT assay, the rate of BrdU incorporation also decreased significantly upon blue light exposure in an intensity-dependent manner ($P < 0.01$; Fig. 2B). In both assays, the inhibitory effect of blue light peaked at 2000 lux and did not increase further even at 5000 lux ($P > 0.05$; Fig. 2A-B). Taken together, blue light significantly inhibits the proliferation of RPE cells in vitro in an intensity-dependent manner.

Blue Light Exposure Downregulated TRPM7 in RPE Cells

Blue light irradiation significantly downregulated the TRPM7 mRNA levels in RPE cells in an intensity-dependent manner, with maximum effects seen at 5000 lux ($P < 0.01$; Fig. 2C). Consistent with this, TRPM7 protein levels were also significantly decreased in the irradiated cells compared to the nonirradiated controls, with the lowest levels seen with 5000 lux ($P < 0.01$; Fig. 2D-E). Therefore, we hypothesized that the effects of blue light on RPE cells were partially mediated by downregulating TRPM7.

TRPM7 Knockdown Aggravated the Effects of Blue Light via PKC/ERK Pathway Blockade

To validate the above hypothesis, we knocked down TRPM7 in RPE cells with the specific siRNA before blue light irradiation. The PKC, ERK, and Bcl-2 protein levels decreased upon photodamage while the Bax protein levels increased, and were restored by 50 ng/mL PEDF ($P < 0.01$; Fig. 3A-B). However, knocking down TRPM7 significantly decreased the protein levels of PKC, ERK, and Bcl-2 ($P < 0.01$; Fig. 3A-B), which increased partially in the presence of PEDF but remained significantly lower compared to the photodamaged cells with regular TRPM7 expression ($P < 0.01$; Fig. 3A). Thus, inhibiting TRPM7 aggravated the effects of blue light. However, pretreatment with 50 ng/mL PEDF partly restored the expression levels of the above proteins in the photodamaged cells ($P < 0.01$; Fig. 3A-D). To determine whether TRPM7 directly affected the proliferation of photodamaged cells, we analyzed BrdU uptake in the differentially treated cells. As shown in Figure 3E, the proliferation rate decreased significantly in the irradiated cells compared to the control. The least BrdU uptake seen in the irradiated TRPM7-knockdown cells, which was assuaged when pretreated with PEDF. Consistent with BrdU uptake, MTT assay showed that the viability of the photodamaged cells with TRPM7-knockdown was the poorest ($P < 0.01$; Fig. 3E), and partly rescued by PEDF ($P < 0.05$; Fig. 3E). Taken together, the absence of TRPM7 aggravates the deleterious effects of blue light by blocking the PKC/ERK pathway.

TRPM7 Overexpression Attenuates the Effects of Blue Light Exposure

To further elucidate the protective role of TRPM7 in photodamaged cells, we overexpressed the protein in RPE cells through a lentiviral system. Interestingly, forced expression of TRPM7 alone (MOI 100) did not completely restore its levels in the blue light–irradiated RPE cells. In the cells pretreated with PEDF, however, the TRPM7 levels increased to 2.28 ± 0.06 compared to the untreated control (1.84 ± 0.05) as well as the blue light–irradiated cells without TRPM7 overexpression (0.52 ± 0.05) ($P < 0.01$; Fig. 4A-B). Consistent with the results so far, overexpression of TRPM7 significantly increased the expression levels of PKC (0.59 ± 0.04) and ERK (1.17 ± 0.05) in the photodamaged RPE cells in the presence of PEDF compared to the blue light–irradiated control (0.06 ± 0.01 and 0.11 ± 0.03 respectively; $P < 0.01$; Fig. 4A-B). The PEDF + vehicle control (MOI 100) group expressed similar levels of the above proteins as PEDF + TRPM7 group ($P > 0.05$; Fig. 4B), indicating that...
PEDF alone has no augmentary effect. Overexpression of TRPM7 and PEDF treatment also increased the expression of Bcl-2 protein ($0.87^{\pm 0.01}$) and decreased that of Bax ($0.63^{\pm 0.01}$) compared to the blue light–irradiated controls ($0.66^{\pm 0.03}$ and $1.03^{\pm 0.03}$ respectively; $P < 0.01$; Fig. 4C-D). Finally, the proliferation rates and metabolic activity of photodamaged RPE cells in the PEDF + TRPM7 and PEDF groups were also higher compared to the blue light control group ($P < 0.01$; Fig. 4E). Taken together, TRPM7 protects RPE cells from blue light–induced apoptosis by activating the PKC/ERK pathway.

**DISCUSSION**

Light radiation via targeted lasers has wide therapeutic applications, for instance, photobiomodulation (PBM) therapy and antimicrobial photodynamic therapy (aPDT). Blue light can also eliminate community-acquired meticillin-resistant *Staphylococcus aureus* (MRSA) from the infected skin abrasions in mice, and alter the inflammatory and immunoregulatory phenotypes of human leukocytes. On the other hand, blue light–induced photochemical damage causes photoreceptor cell death, the severity of which depends on the light intensity, exposure time, and wavelength.

Previous studies have shown that the effect of blue light on the retina depends on the frequency bands. In addition, blue light exposure induced apoptosis in RPE cells in vitro in duration- and intensity-dependent manner, and 6-hour exposure to 2000 lux is the optimum condition for triggering photodamage in human RPE cells. In this study, we successfully established an in vitro blue light damage model by irradiating rodent RPE cells with the light of wavelength range 440–10 nm, and the optimum conditions were as previously described. Blue light exposure significantly decreased the metabolic activity and proliferation of RPE cells in an intensity-dependent manner. The pathological basis of photoreceptor damage of retinal cells is light-induced apoptosis accompanied by calcium disturbance, mitochondrial damage, metabolic abnormalities, and oxidative damage. Downregulation of TRPM7 induced apoptosis in human bladder cancer cells via the ERK1/2 pathway and disrupted the Bax/Bcl-2 ratio in skin fibroblasts leading to apoptosis. In addition, blocking TRPM7 channels through the reticular stress-mediated pathway induced apoptosis in hepatic stellate cells. In our study also, we found that blue light irradiation downregulated TRPM7, PKC/ERK, and Bcl-2 protein levels in the RPE cells, and upregulated Bax. The decreased metabolic activity of RPE cells exposed to blue light correlated to low TRPM7 levels. In contrast,
FIGURE 3. Effect of TRPM7 siRNA on the TRPM7, PKC/ERK, Bax/Bcl-2 protein levels and the proliferation and metabolic viability (OD value) of photodamaged RPE cells. Group blue light was exposed to 2000 lux of blue light for 6 h, and cultured thereafter for 24 h. Group PEDF, group PEDF + scrambled RNA and group PEDF + TRPM7 siRNA were cultured with 50 ng/mL PEDF, 50 ng/mL PEDF + scrambled RNA 20 ng and 50 ng/mL PEDF + TRPM7 siRNA 20 ng respectively, then were all exposed to the same illumination conditions as the blue light group. $^a$P < 0.01 compared with the control group, $^b$P < 0.05 compared with group blue light. Bax indicates Bcl2-associated x; Bcl-2, B-cell lymphoma 2; ERK, extracellular signal-regulated kinase; PEDF, pigment epithelium-derived factor; PKC, protein kinase C; RPE, retinal pigment epithelium; siRNA, small interfering RNA; TRPM7, transient receptor potential melastatin 7.

FIGURE 4. Effect of TRPM7 lentivirus on the TRPM7, PKC/ERK, Bax/Bcl-2 protein levels and the proliferation and metabolic viability (OD value) of photodamaged RPE cells. Group blue light were exposed to 2000 lux of blue light for 6 h, and cultured thereafter for 24 h. Group TRPM7, group PEDF, group PEDF + TRPM7 lentivirus were interfered with TRPM7 lentivirus (MOI 100), 50 ng/mL PEDF, 50 ng/mL PEDF + TRPM7 lentivirus (MOI 100) respectively, then were all exposed to the same illumination conditions as the blue light group. $^a$P < 0.01 compared with the control group, $^b$P < 0.05, $^c$P < 0.01 compared with group blue light. Bax indicates Bcl2-associated x; Bcl-2, B-cell lymphoma 2; ERK, extracellular signal-regulated kinase; PEDF, pigment epithelium-derived factor; PKC, protein kinase C; RPE, retinal pigment epithelium; TRPM7, transient receptor potential melastatin 7.
overexpression of TRPM7 increased PKC/ERK and Bcl-2 expression levels and restored proliferation of the photodamaged RPE cells. We hypothesized, therefore, that TRPM7 promotes survival of photodamaged RPE cells via the PKC/ERK pathway, and is a potential therapeutic target for light-induced retinopathies.

PEDF is a pleiotropic neurotrophic factor and neovascular inhibitor that is widely distributed in the human body. Previous studies have shown that PEDF protects retinal cells against photodamage, oxidative stress injury and ischemia-reperfusion injury, and plays an important role in the growth and development of retinal tissue, and retinal cell differentiation and maintenance. Cao et al had shown that intravitreal injection of 2 μl PEDF before 1200–1500 lux of light exposure protected the retinal photoreceptors against photodamage, and its protective effect was superior to that of brain-derived neurotrophic factor (BDNF). Pretreatment with PEDF protects retinal neurons from H2O2-induced apoptosis in a dose–response relationship with a half effective dose of 50 ng/ml. In an in vitro oxidative damage model of RPE cells (ARPE-19 cell line), treatment with 5 nM and 10 nM PEDF reduced NaO3-induced cytotoxicity by 50%. In addition, the protective effect of PEDF on photoreceptor cells is related to the calcium-mediated PKC pathway. In this study, PEDF restored the proliferation and metabolic activity of light-damaged RPE cells and increased their survival by reversing the photodamage-induced inhibition of PKC ERK and Bcl-2 levels at the half effective dose of 50 ng/mL.

Knocking down TRPM7 decreased the prosurvival effects of PEDF, while overexpression of TRPM7 synergistically increased the proliferation of RPE cells in the presence of blue light. This synergistic protective effect is likely related to the PKC/ERK pathway. There is also a possibility of crosstalk between the individual signal transduction pathways activated by PEDF and TRPM7, which form a complex signaling network regulating cell growth, proliferation, differentiation, and photodamage repair, although the underlying mechanisms need to be resolved.

To summarize our findings, TRPM7 and PEDF synergistically protected RPE cells against blue light–induced damage involving PKC/ERK signaling pathway. However, we only focused on one possible mechanism of TRPM7, although it is involved in multiple physiological and pathological processes. Further studies will have to elucidate other regulatory mechanisms of RPE photodamage. TRPM7 is a pleiotropic protein that plays different regulatory roles depending on the cell type and differentiation status. The same signaling pathway activated by TRPM7 may have different functions in different cells. Therefore, the specific pathway utilized by TRPM7 in RPE cells needs to be clarified. TRPM7 also plays an essential role in the regulation of calcium homeostasis, which is critical to the physiological function of RPE cells. It is essential to explore the potential role of TRPM7-mediated Ca2+ and Mg2+ influx in the photodamaged RPE cells and determine any possible crosstalk with PEDF or other factors.

In addition, there are some limitations of our study. Firstly, there was no positive control and further research is needed to compare the light exposure effect between blue light and other light. Secondly, we used SD rat which is nonpigmented and the use of other pigmented species might yield different results. Thirdly, this was only an in vitro model and further in vivo animal studies are required to confirm the findings.

Nevertheless, our findings provide the experimental basis for further studies on the mechanisms of blue light–induced apoptosis of RPE cells, as well as new insights into the pathogenesis, prevention, and treatment of various retinopathies.

CONCLUSIONS
Blue light triggers apoptosis of RPE cells in an intensity-dependent manner. TRPM7 and PEDF show potential protective effect synergistically of RPE cells from photodamage via the PKC/ERK signaling pathway.

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