TRPM7 ameliorates the blue light-induced apoptosis of RPE cells involving PKC/ERK

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Research

Keywords: Light-induced damage, Retinal pigmented epithelium, TRPM7, apoptosis, signal pathway

Posted Date: February 25th, 2020

DOI: <https://doi.org/10.21203/rs.2.24470/v1>

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Abstract

Background 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 TRPM7 in photo-damaged RPE cells.

Methods RPE cells isolated from Sprague-Dawley (SD) rats were cultured in vitro, and exposed to varying intensities of blue light (500-5000 Lux). Cell proliferation and viability were respectively assessed by BrdU incorporation and MTT assays. Real-time PCR and Western blotting were used to analyze the mRNA and protein expression levels of TRPM7, PKC, ERK and Bax/Bcl-2. The cells were transfected with TRPM7 siRNA to knockdown its mRNA levels, or transduced with TRPM7-overexpressing lentiviruses. Pigment epithelium-derived factor (PEDF) was used in combination to detect the anti-apoptosis effect.

Results Blue light inhibited the proliferation and viability of RPE cells in an intensity-dependent manner when compared to non-irradiated controls ($P < 0.05$). Compared to the control, photo-damaged RPE cells showed decreased levels of TRPM7, PKC, ERK and Bax, increased in Bcl-2 ($P < 0.01$). Forced expression of TRPM7 partially ameliorated the reduction of proliferation and viability of RPE cells ($P < 0.01$), alleviated the downregulation of TRPM7, PKC, ERK and Bax expression levels ($P < 0.01$), induced by blue light irradiation, while TRPM7 knockdown had opposite effects ($P < 0.01$). TRPM7 and PEDF synergistically alleviated the damaging effects of blue light.

Conclusions The apoptosis of RPE cells induced by blue light was positively correlated with the expression of TRPM7. Forced expression of TRPM7 partially attenuated the deleterious effects of blue-light-damaged RPE cells and showed a synergistic protective effect with PEDF, involving PKC/ERK signaling pathway.

Background

Light is a double-edged sword for the visual system. It is essential for visual perception, but can adversely affect the eyes under certain conditions [1]. An appropriate amount of blue light is necessary to regulate the circadian rhythm of human body [2] and can also affect the development of myopia [3], while excessive exposure could lead to dry eyes [4], visual fatigue, photo-damage to the cornea, lens and retina, and even retinal degeneration leading to irreversible visual damage [5]. Otherwise, retinal photodamage and the apoptosis of RPE cells precedes the development of various retinopathies [6]. Therefore, it is essential to explore the mechanisms underlying blue light-mediated retinal damage and develop protective measures.

Apoptosis of RPE cells caused by blue light plays an essential role in retinal photodamage, such as calcium homeostasis, mitochondrial damage, metabolic abnormalities, and oxidative damage [7]. The mitogen-activated protein kinase (MAPK) signaling pathway regulates multiple processes such as cell proliferation, survival, differentiation and apoptosis, and is activated by upstream kinases like the protein

kinase C (PKC) [8, 9]. A study about the role of fucoidan in diabetic retinopathy (DR) indicated that fucoidan could protect ARPE-19 cells against high glucose-induced oxidative damage involving the Ca^{2+} -dependent ERK signaling pathway [10]. Calcium ions play a critical role in neuronal cell death. A previous study on photo-damaged human RPE cells showed that the Ca^{2+} /PKC signaling pathway regulated the expression of photo-protective pigment epithelium-derived factor (PEDF) and the signaling intermediates inositol triphosphate (IP3) and diacylglycerol (DAG) through a feedback loop [11].

TRPM7 is a member of the Transient Receptor Potential (TRP) channel superfamily, which is widely expressed in mammalian heart, liver, lung, kidney, and retina [12, 13]. TRPM7 is a bifunctional protein with a dual structure of ion channels and protein kinases. TRPM7 channel kinase is an essential cellular sensor and physical and chemical stress sensor that plays a vital role in a variety of biological processes, physiological functions and embryonic development [14]. TRPM7 also plays an important role in the regulation of calcium homeostasis, which is critical to the physiological function of RPE cells and regulates the calcium channel and PKC/ERK signaling pathway upon blue light injury. Many studies showed that TRPM7 played a neuroprotective role in the ischemic brain injury and oxygen-glucose deprivation (OGD) model, and during oxidative stress and inflammation. However, the pathways underlying the neuroprotective, proliferative and antioxidant effects of TRPM7 remain to be elucidated. It is, however, unclear whether TRPM7 plays a direct protective role in photo-damaged RPE cells.

In this study, we established an in vitro blue-light damage model using RPE cells isolated from Sprague-Dawley (SD) rats and observed significant downregulation in TRPM7, PKC, ERK, and Bcl-2 levels in an intensity-dependent manner. Forced expression of TRPM7 partially attenuated the deleterious effects of blue light on the RPE cells and showed a synergistic protective effect with PEDF. Our findings provide new insights into the pathogenesis, prevention and potential treatment of retinal photodamage and other different retinopathies.

Methods

Cell culture and blue light-induced RPE cell injury

Rat RPE cells (CHI Scientific, Inc.) were cultured in DMEM/F12 media containing 10% FBS and 1% penicillin/streptomycin (all from Gibco) at 37°C and 5% CO_2 . The media was changed every two days, and once the cells reached 90% confluency. All animal procedures were conducted following the guidelines of the Association for Research in Vision and Ophthalmology (ARVO) Statement on the Use of Animals in Ophthalmic and Vision Research. The RPE cells cultured in vitro were randomly divided into group A that were respectively exposed to varying intensities (0, 500, 1000, 2000, and 5000 Lux) and group B that were exposed to 2000 Lux of blue light for 6h, and cultured after that for 24h. An unexposed control group was also included. During exposure, the cells and the blue light tube (440 ± 10 nm, 20 W) were placed inside the CO_2 incubator, and the latter was mounted on a special framework. The TES1332A illuminometer was used to measure the light intensity at the cell surface, and the distance between the tube and the cell surface was adjusted according to light intensity. The temperature of the incubator is

controlled between 37 °C and 38 °C. The proliferation of RPE cells was determined using MTT Cell Proliferation kit as per the manufacturer's instructions, as well as by BrdU incorporation assay. The absorbance of samples was measured using PerkinElmer at 450 nm.

Real-time polymerase chain reaction (RT-PCR)

Total RNA was isolated from RPE cells using RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol and treated with RNase free DNase to eliminate genomic DNA. Two micrograms total RNA per sample was reverse transcribed into cDNA using the PrimeScript® 1st strand cDNA Synthesis Kit (Takara). RT-PCR was performed on an Agilent Stratagene Mx3000P QPCR Systems using SYBR® Premix Ex Taq™ Kit (Takara). The cDNA samples were stored at -20 °C for future analysis.

All primer design and synthesis were performed by TaKaRa. The primer sequences are as follows:

The forward primer of TRPM7: 5'-AGTATATCGTCTGGAGGAGAGTTC-3'; reverse primer of TRPM7: 5'-ATTTGGGTTTCATCTGATTAAAGGC-3'; forward primer of GAPDH: 5'- CTCATGACCACAGTCCATGCCA -3'; reverse primer of GAPDH: 5'- GCCTTGGCAGCACCAGTGGATG -3'.

The expression of the target gene was calculated relative to the expression of the internal reference gene, GAPDH.

Western Blotting

Total protein was extracted from RPE cells using RIPA buffer supplemented with PMSF at the ratio of 100:1 and quantified using the BCA assay. Equal amounts of protein per sample were separated through a 10%–15% SDS-PA gel, and the bands were transferred onto polyvinylidene difluoride (PVDF) membranes according to the manufacturer's protocol. After blocking with a commercially available blocking buffer (Beyotime, Shanghai, China), the protein blots were incubated overnight with primary antibody (rabbit anti-TRPM7, rabbit anti-PKC, rabbit anti-ERK and rabbit anti-GAPDH primary antibodies Abcam) at 4°C.

SiRNA Transient Transfection

TRPM7 small interfering RNA (siRNA) and Scrambled RNA (control siRNA) were purchased from Sigma Biotechnology (Sigma, USA). The siRNAs were transfected into RPE cells according to the manufacturer's instructions using the Lipofectamine®RNAiMAX transfection reagent (Invitrogen, USA). RPE cells at 3rd–6th passages were isolated with 0.25% Trypsin and seeded in 6-well plates at a density of 1×10^5 cells/well containing DMEM/F12 with 10% FBS. When cells grow to 60%–70% confluence, the medium was replaced by serum-free DMEM/F12 for 24 hours and incubated with 20 nM Scrambled or TRPM7 siRNA for 6 h in serum-free OPTI-MEM media (Invitrogen, USA). After the incubation, the transfected media was replaced by serum-free DMEM/F12 medium for using subsequent experiments.

TRPM7 lentivirus Transient transfection

Cells in exponential growth phase were plated in 6-well plates at a density of 1×10^5 cells/well. When cells grow to 60%–70% confluence, the medium was replaced by serum-free DMEM/F12 for 24 hours and incubated with Vehicle(control lentivirus) or TRPM7 lentivirus for 24 h in serum-free OPTI-MEM media. After 24 hours, the transfection medium was replaced by serum-free DMEM/F12 medium for using subsequent experiments

Statistical Analysis

Statistical analysis was performed using the SPSS software (version 19.0), and the data were presented as mean $\pm$ SEM. 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 (a–c), Positive rates of CK 18 expressions in RPE cells 3rd generation under fluorescence microscope. Representative fluorescence images showing CK-18 positive (green, Figure 1a) cells counterstained with DAPI (blue, Figure 1b); More than 95% of the cultured RPE cells were positive for CK-18 (Figure 1c), which is the typical epithelial surface markers, and showed the typical hexagonal, polygonal or fusiform morphology. The cells 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 viability of RPE cells in an intensity-dependent manner compared to the non-irradiated control ($P < 0.01$; Figure 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 ($P < 0.01$; Figure 2b). In both assays, the inhibitory effect of blue light peaked at 2000 Lux, and did not increase further even at 5000 Lux ($P > 0.05$; Figure 2a, 2b). 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 ($P < 0.01$; Figure 2c). Consistent with this, TRPM7 protein levels were also significantly decreased in the irradiated cells compared to the non-irradiated controls, with the lowest levels seen with 5000 Lux ($P < 0.01$; Figure 2d, 2e). Therefore, we hypothesized that the effects of blue light on RPE cells were mediated by downregulating TRPM7.

TRPM7 knockdown downregulated PKC/ERK, Bcl-2 and decreased proliferation of photo-damaged RPE cells

To validate the above hypothesis, we knocked down TRPM7 in RPE cells with the specific siRNA prior to blue light irradiation. As shown in figure 3, the TRPM7, PKC, ERK and Bcl-2 protein levels were decreased to (0.34 ± 0.05) , (0.11 ± 0.02) , (0.13 ± 0.02) , (0.38 ± 0.01) in group PEDF+TRPM7 siRNA compared to control group (2.33 ± 0.08) , (0.74 ± 0.07) , (0.73 ± 0.08) , (0.91 ± 0.02) ($P < 0.01$, Figure 3a-d), while Bax protein levels were increased from (0.55 ± 0.02) in control group to (1.01 ± 0.02) ($P < 0.01$; Figure 3a-d). The TRPM7, PKC, ERK, Bcl-2 protein levels decreased upon photodamage while the Bax protein levels increased, and were restored by 50 ng/ml PEDF ($P < 0.01$, Figure 3a-d). Thus, inhibiting TRPM7 aggravated the effects of blue light.

To determine whether TRPM7 directly affected the proliferation of photo-damaged 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, with the least BrdU uptake seen upon TRPM7 knockdown, which was assuaged when treated with PEDF. Consistent with the results of the BrdU uptake assay, MTT assay showed the lowest viability in the photo-damaged cells with TRPM7-knockdown ($P < 0.01$, Figure 3e), and treatment with PEDF partly rescued the cells ($P < 0.05$, Figure 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 photo-damaged cells, we overexpressed the protein in RPE cells through a lentiviral system. Interestingly, forced expression of TRPM7 (TRPM7 lentivirus, MOI 100) did not completely restore its levels in blue light-irradiated RPE cells. However, in the PEDF-treated cells, TRPM7 levels were expectedly increased (2.28 ± 0.06) in group PEDF + TRPM7 lentivirus compared with control group (1.84 ± 0.05) and blue light group (0.52 ± 0.05) ($P < 0.01$; Figure 4a-b). Consistent with the results far, overexpression of TRPM7 significantly increased the expression levels of PKC (0.59 ± 0.04) and ERK (1.17 ± 0.05) in the photo-damaged RPE cells in group PEDF + TRPM7 lentivirus compared with blue light group (0.06 ± 0.01) and (0.11 ± 0.03) ($P < 0.01$, Figure 4a-b), and PEDF treatment had no significant additional effect compared to the group PEDF + TRPM 7 vehicles (MOI 100) ($P > 0.05$; Figure 4b).

Otherwise, overexpression of TRPM7 also increased the expression of Bcl-2 protein levels, (0.87 ± 0.01) in group PEDF + TRPM7 lentivirus and (0.66 ± 0.03) in group blue light, while decreased in Bax, (0.63 ± 0.01) in group PEDF + TRPM7 lentivirus and (1.03 ± 0.03) in group blue light ($P < 0.01$, Figure 4c-d). Finally, the proliferation and viability of photo-damaged RPE cells in group PEDF + TRPM7 lentivirus and group PEDF were also increased compared to group blue light ($P < 0.01$, Figure 4e). Taken together, TRPM7 plays a protective role in blue light-exposed RPE cells, reduces apoptosis and restores their proliferation levels by activating the PKC/ERK pathway.

Discussion

Light-induced photochemical damage causes photoreceptor cell death, the severity of which depends on the light intensity, exposure time, and wavelength [15, 16]. Previous studies showed that blue light exhibits two-sidedness according to different frequency bands—harmful blue light (415–460 nm) and beneficial

blue light (460–480 nm) [16]. So we used blue light-shielding films with different shielding rates of wavelength (440 ± 10) nm to establish an in vitro blue-light damage model and explore the protective role of TRPM7 in photo-damaged RPE cells.

In this study, we found that the viability and proliferation of RPE cells decreased in an intensity-dependent manner when exposed to blue light (0–5000 Lux) and observed significant downregulation in TRPM7, PKC, ERK and Bcl-2 protein levels in an intensity-dependent manner. Previous studies showed that blue light exposure induced apoptosis in RPE cells in vitro in duration and intensity-dependent manner, and that 6h exposure to 2000 Lux were the optimum conditions for establishing a blue-light injury model of human RPE cells in vitro [6, 11]. In our study, we successfully established an in vitro model of photo-damage by exposing RPE cells from SD rats to the same conditions. We found that 6h exposure to varying intensities (0–5000 Lux) of blue light significantly decreased the proliferation and viability of RPE cells, with maximum inhibitory effects seen at 2000 and 5000 Lux. Our study demonstrated that the best conditions to establish a blue light damage model of rat RPE cells in vitro were 2000Lux light intensity for 6h and established a blue light damage model of rat RPE cells in vitro. Our findings will provide the experimental basis for future studies on the mechanisms of blue light-induced apoptosis of RPE cells and provide new insights into the pathogenesis, prevention and potential treatment of different retinopathies.

Previous studies have suggested that light-induced apoptosis of retinal cells is the pathological basis of photoreceptor damage in retinal cells, which is accompanied by calcium disturbance, mitochondrial damage, metabolic abnormalities, and oxidative damage [17]. Down-regulation of TRPM7 induced apoptosis in human bladder cancer cells via ERK1/2 pathway and disrupted the Bax/Bcl-2 ratio in skin fibroblasts leading to apoptosis. Besides, blocking TRPM7 channels through the reticular stress-mediated pathway induced apoptosis in hepatic stellate cells [18]. In our study also, we found that blue light irradiation downregulated TRPM7, PKC/ERK, and Bcl-2 protein levels in RPE cells, and up-regulated the expression of Bax. The decreased viability of RPE cells exposed to blue light was correlated to low TRPM7 levels. In contrast, overexpression of TRPM7 increased PKC/ERK and Bcl-2 expression levels and restored proliferation of photo-damaged RPE cells. We hypothesized, therefore that TRPM7 regulates survival of photo-damaged RPE cells through PKC/ERK pathway, and can be a potential target for treating light-induced retinopathies.

PEDF is a pleiotropic neurotrophic factor and neovascular inhibitor that is widely distributed in human body [19]. Previous studies have shown that PEDF protects retinal cells against photodamage [20], 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 [21, 22]. Cao W and Tombran-Tink J et al [23] had shown that intravitreal injection of 2 μ l PEDF has a protective effect on retinal photoreceptors, 1 to 2 days before light exposure (light intensity range from 1200Lux to 1500Lux), whose protective effect is superior to brain-derived neurotrophic factor(BDNF). Pretreatment with PEDF protects retinal neurons from H_2O_2 -induced apoptosis in a dose-response relationship with a half effective dose of 50 ng/ml [22]. In an oxidative damage model of RPE cells (ARPE-19 cell line) cultured in vitro, adding 5nM and 10nM PEDF could reduce about 50% cytotoxicity induced by $NaIO_3$ [24]. In addition,

the protective effect of PEDF on photo retina is related to the calcium-mediated PKC pathway [11]. In this study, we used half effective dose of 50 ng/ml of PEDF. PEDF restored the proliferation and viability of light-damaged RPE cells and increased their survival by reversing the photodamage-induced inhibition of PKC ERK and Bcl-2 levels.

Knockdown of TRPM7, on the other hand, decreased the pro-survival 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 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 photo-damage 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, whereas it is involved in multiple physiological and pathological processes. Further studies will have to elucidate other regulatory mechanisms of RPE photo-damage. 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 Ca^{2+} and Mg^{2+} influx in the photo-damaged RPE cells and determine any possible crosstalk with PEDF or other factors. Nevertheless, our findings provide novel insights into the treatment of retinopathies and justify further mechanistic and functional studies.

Conclusions

The apoptosis of RPE cells induced by blue light exposure was correlated with TRPM7 gene involving PKC/ERK signaling pathway. Overexpression of TRPM7 partially attenuated the apoptosis of blue-light-damaged RPE cells, and showed a synergistic protective effect with PEDF.

Declarations

Ethics approval and Consent to participate

All animal procedures were conducted following the guidelines of the International Council for Laboratory Animal Science (ICLAS) and the Association for Research in Vision and Ophthalmology (ARVO) Statement on the Use of Animals in Ophthalmic and Vision Research.

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by the national natural science foundation of China (No.81271026 & No.81770948).

Authors' contributions

Luping Hu designed the study, performed the experiments, analyzed the data and wrote the manuscript. Guoxing Xu directed the works and reviewed the manuscript. Both authors have read and approved the final manuscript.

Acknowledgements

Not applicable

Abbreviations

TRP: Transient Receptor Potential

RPE: retinal pigment epithelium

PEDF: pigment epithelium-derived factor

PKC: protein kinase C

SD: Sprague-Dawley

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Figures

Figure 1

Characterization of RPE cells cultured in vitro. Positive rates of CK 18 expressions in RPE cells (3rd generation) under fluorescence microscope. Positive rates of CK 18 expressions in RPE cells were obtained as follows: 5 fields per sample were chosen randomly under a fluorescence microscope at 200× magnification. Representative fluorescence images showing CK-18 positive (green, Figure 1a) cells counterstained with DAPI (blue, Figure 1b); Figure 1c. More than 95% of the cultured RPE cells were positive for CK-18. The cells were relatively uniform in shape and arranged in a "paved stone" manner.

Figure 2

Blue light exposure decreased the proliferation and downregulated TRPM7 of RPE cells. A. The results of the MTT assay showed that the viability of the RPE cells exposed to blue light (440 ± 10 nm, $0-5000$ Lux) significantly decreased in an intensity-dependent manner compared to the non-irradiated control. B. The BrdU incorporation rate of RPE cells decreased in an intensity-dependent manner when exposed to blue light. C. Effect of different light intensities on TRPM7 mRNA levels in RPE cells. TRPM7 mRNA/GAPDH mRNA ratio expressed TRPM7 mRNA expression level, repeated 5 times for each experiment ($n = 8$). D.

Effects of different light intensities on TRPM7 protein levels in RPE cells. Western blotting, group1-Control group, group2-500lux, group3-1000lux, group4-2000lux, group5-5000lux. E. Analysis of TRPM7 protein levels in terms of TRPM7/ β -actin ratio. **P < 0.01 compared with the control group. ##P < 0.01 compared with the group 3 (1000 Lux).

Figure 3

Effect of TRPM7 siRNA on the TRPM7, PKC/ERK, Bax/Bcl-2 protein levels and the proliferation and viability of photo-damaged RPE cells. Group Blue Light were exposed to 2000 Lux of blue light for 6h, and cultured thereafter for 24h. Group PEDF→group PEDF + Scramble RNA and group PEDF+TRPM7 siRNA were cultured with 50ng/ml PEDF →50ng/ml PEDF + Scramble RNA 20ng and 50ng/ml PEDF + TRPM7 siRNA 20ng respectively→then were all exposed to the same illumination conditions as the Blue Light group. **P < 0.01 compared with the control group. ##P < 0.01→#P < 0.05 compared with the group Blue Light.

Figure 4

Effect of TRPM7 lentivirus on the TRPM7, PKC/ERK Bax/Bcl-2 protein levels and the proliferation and viability of photo-damaged RPE cells. Group Blue Light were exposed to 2000 Lux of blue light for 6h, and cultured thereafter for 24h. Group TRPM7, group PEDF, group PEDF + TRPM7 lentivirus were interfered with TRPM7 lentivirus (MOI 100), 50ng/ml PEDF, 50ng/ml PEDF + TRPM7 lentivirus (MOI 100) respectively, then were all exposed to the same illumination conditions as the Blue Light group. **P < 0.01 compared with the control group. #P < 0.05, ##P < 0.01 compared with the group Blue Light.