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Protective effect of chitosan oligosaccharides on blue light light-emitting diode induced retinal pigment epithelial cell damage

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ABSTRACT

Excessive blue light light-emitting diode (LED) exposure and consequent oxidative stress causes damage to retinal pigment epithelial (RPE) cells. Chitosan oligosaccharides (COSs) are the hydrolyzed products of chitin, which is abundant in the exoskeleton of crustaceans and cell walls of fungi. We investigated the protective effect of COSs on blue light LED-induced RPE cell damage. These cells were treated with various concentrations of COSs and then exposed to blue light LED. Our results confirmed that RPE cell apoptosis increased significantly with longer light exposure. Treatment with COSs significantly reduced apoptosis in a dose-dependent manner. These molecules also suppressed the production of reactive oxygen species and the expression of inflammation- and apoptosis-related proteins. Moreover, COSs stabilized the mitochondrial membrane potential of cells and down-regulated the NF-κB pathway. In this study, the protective effects of COSs on blue light LED-induced RPE cell damage, as well as the associated mechanisms, were demonstrated.

1. Introduction

Artificial lighting is a common element of modern society; however, the potential health risks caused by light pollution have increased with the development of more sophisticated lighting technology (Chepesiuk, 2009). Among the wide variety of artificial lighting selections, light-emitting diodes (LEDs) emit higher levels of blue light than conventional light sources. These have provided humans with their first exposure to such extensive blue light (Behar-Cohen et al., 2011). Blue light LED and white light LED are now widely used in illumination light, flashlights, digital display, and the screens of computers, smartphones, or other consumer electronics. In the eye, wavelengths from the spectral band of 400–1400 nm can reach the retina (Boettner & Wolter, 1962). The retina contains specific chromophores in the photoreceptor that function to absorb visible radiation. The main sites of light energy absorption are the melanin pigment granules in retinal pigment epithelial (RPE) cells, which can be injured by light exposure (Parver, Auker, & Fine, 1983; Tso, 1973).

It is generally accepted that lights with shorter wavelengths are significantly more hazardous than those with longer wavelengths; as such, blue light-induced RPE cell damage has been reported (Boulton, Rozanowska, & Rozanowski, 2001; Chamorro et al., 2013; Dorey, Delori, & Akeo, 1990; Godley et al., 2005; Ham, Mueller, & Sliney, 1976). Epidemiological studies have suggested that short-wavelength light exposure is a predisposing factor for age-related macular degeneration (AMD) (Wu, Seregard, & Algvere, 2006). Previous studies have also demonstrated that exposure to blue and white light can induce damage to photoreceptors and RPE cells of the rat retina (Hafezi, Marti, Munz, & Reme, 1997; Shang, Wang, Sliney, Yang, & Lee, 2014). We also previously found that the mechanism of retinal injury is related to oxidative stress within the retina (Shang, Wang, Sliney, Yang, & Lee, 2017), which can induce the generation of reactive oxygen species (ROS) and inflammatory reactions (Hollyfield et al., 2008; Zhou, Jang, Kim, & Sparrow, 2006). Since RPE cells are the major ocular source of pro-inflammatory mediators and the primary target of photo-oxidative effects, oxidative damage to these cells might contribute to ocular inflammation and AMD-related lesions.

Nutritional supplements are widely taken by the general population and several of these products are marketed specifically for the improvement of eye health. The Age-Related Eye Disease Study (AREDS), a randomized clinical trial, showed a significantly lower incidence of late AMD in a cohort of patients with drusen maculopathy treated with high

Abbreviations: LED, light-emitting diode; RPE, retinal pigment epithelia; COS, chitosan oligosaccharides; AMD, age-related macular degeneration; ROS, reactive oxygen species; AREDS, Age-Related Eye Disease Study; PI, propidium iodide; DCFH, dichlorodihydrofluorescein; HO-1, heme oxygenase-1; iNOS, inducible nitric oxide synthase; Bcl-2, B-cell lymphoma 2; HRP, horseradish peroxidase; MCP-1, monocyte chemoattractant protein-1

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doses of antioxidants, compared to that in a placebo group (Chiu & Taylor, 2007; Robman et al., 2007). In addition, sulforaphane, a compound found in cruciferous vegetables, has been shown to protect RPE cells from photo-oxidative damage (Gao & Talalay, 2004). Polyphenols extracted from bilberry and lingonberry also protect retinal photoreceptor cells from blue light LED-induced damage (Ogawa et al., 2014).

Chitosan oligosaccharides (COSs), the hydrolyzed products of chitin, consist of a mixture of oligomers of β-1,4-linked D-glucosamine residues. Chitin is abundant in the exoskeleton of crustaceans and the cell walls of fungi and insects (Pae et al., 2001). COSs are known to exert various biological effects including anti-tumor, anti-bacterial, anti-inflammation, anti-oxidative, and anti-apoptotic activities (Chen, Liu, Du, Peng, & Sun, 2006; Joodi, Ansari, & Khodagholi, 2011; Pangestuti & Kim, 2010; Qin, Du, Xiao, Li, & Gao, 2002). Importantly, COSs are non-toxic and biodegradable and have thus been used as bioactive material. In addition, they have good solubility in water and are easily absorbed in the intestine, which makes them an attractive ingredient in many healthy foods or dietary supplements. We have previously shown that COSs exert their anti-oxidative effects by inhibiting NF-κB activation and by attenuating retinal oxidative stress-related retinal degeneration in rats (Fang, Yang, Yang, & Chen, 2013). Recently, we also demonstrated the protective effect of COSs on retinal ischemia and reperfusion injury, which was shown to occur via the inhibition of oxidative stress and inflammatory mediators (Fang, Yang, & Yang, 2015).

The increasing popularity of the consumer electronics brings convenience to our lives; however, most of these products use LED as their light source and thus emit blue light. Since COSs have anti-apoptotic and anti-oxidative effects, we investigated the protective effects of these molecules on blue light LED-induced RPE cell damage and evaluated the potential of COSs to be used as a nutrient supplement for the prevention of blue light-mediated damage to the human eye.

2. Material and methods

2.1. Human ARPE-19 cell culture

ARPE-19 cells were purchased from the American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) with 10% fetal bovine serum, 4.5 mg/mL glucose, 100 units/mL penicillin, and 100 μg/mL streptomycin in a humidified incubator at 37 °C in an atmosphere of 5% CO_2. ARPE-19 cells were seeded at a density of 1×10^4 cells per well in plates. These cells were passaged by trypsinization every 3–4 d. Cells were used at the third to fifth passages.

2.2. Blue light LED and COSs treatment of RPE cells

ARPE-19 cells were exposed to blue light LED with an intensity of 2500 lx; cells were cultured for 24–48 h after exposure. A medical blue light LED tube (450 ± 20 nm, 20 W) was placed on a special framework, and the illuminometer was used to measure light intensity at the cell surface. The distance between the tube and the cell surface was adjusted according to light intensity. To ensure a stable growth environment for RPE cells, the blue light device was placed inside the CO_2 incubator. Temperature was maintained between 36.5 and 37.5 °C. COSs were purchased from Sigma-Aldrich (catalog number 523682, St. Louis, MO, USA). The average molecular weight of COSs was 5000 and the degree of N-deacetylation was more than 90%. Cells were treated with COSs at various concentrations (ranging from 50 to 200 μM) and then exposed to blue light LED. COSs remained in the medium during LED exposure.

2.3. Analysis of apoptosis by flow cytometry

The proportion of ARPE-19 cells undergoing apoptosis was determined at 24 or 48 h after exposure by flow cytometry using Annexin-V and propidium iodide (PI) staining. The staining solution contained 5 μL Annexin-V-FITC in 250 μL binding buffer and 5 μL PI (Strong Biotech, Taiwan). The ARPE-19 cells were washed with PBS and centrifuged at 200*g* for 5 min. Then, the cell pellet was resuspended in 100 μL of staining solution and incubated for 10 min at 20 °C. Finally, the sample was added to 900 μL binding buffer and analyzed using a FACScan cytometer (BD Bioscience, NJ, USA).

2.4. Cell viability assay

Cell viability was determined using the alamar blue assay (Thermo Fisher Scientific, MA, USA). ARPE-19 cells were cultured in 96-well plates, and then incubated for 24 h in a humidified atmosphere of 5% CO_2 at 37 °C. We added 500 μL of alamar blue to each well. Cells were treated with COSs at various concentrations (0, 50, 100, 200 μM) for 1 h. Then, the cells in the exposure group were exposed to blue light LED for 24 h. The absorbance was measured at a wavelength of 570 nm and 600 nm using microplate reader (Bio-Rad Laboratories Inc., CA, USA).

2.5. Detection of intracellular ROS

Intracellular ROS levels were measured using 2′,7′-dichlorodihydrofluorescein diacetate (2′,7′-DCFDA, Sigma-Aldrich, St. Louis, MO, USA) oxidation. ARPE-19 cells were exposed to 2500 lx of blue light LED for 48 h after being treated with different concentrations of COSs (0, 25, 100 μM) for 1 h. Then, 10 mM of 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H2DCFDA), a free radical probe, was added to the cell and incubation was continued for 1 h at 37 °C. The radical probe was converted to 2′,7′-dichlorodihydrofluorescein (DCFH) by the action of intracellular esterase. Intracellular DCFH (non-fluorescent) was oxidized to 2′,7′-dichlorfluorescein (DCF, fluorescent) by intracellular ROS. Fluorescence was measured using a microplate reader (Bio-Rad Laboratories Inc., CA, USA) at 485 nm (excitation) and 535 nm (emission).

2.6. Determination of mitochondrial dysfunction

The mitochondrial membrane potential was measured using the JC-1 Mitochondrial Membrane Potential Assay Kit (Cayman Chemical Company, Ann Arbor, MI). Different concentrations of COSs (0, 50, 100, 200 μM) were added to the cells exposed to blue light LED. After 24 h of 2500-lux blue LED light exposure, 50 μL of JC-1 staining buffer was added to 1 mL of culture medium. JC-1 formed J-aggregates in healthy cells and remained in its monomeric form in apoptotic or unhealthy cells. J-aggregates and JC-1 monomers were detected with settings designed to detect Texas Red and FITC, respectively. The images were captured using a fluorescence microscope, which was used to detect healthy cells with mainly JC-1 J-aggregates (excitation/emission, 540/605 nm) and apoptotic or unhealthy cells with mainly JC-1 monomers (excitation/emission, 480/510 nm). The number of cells (red or green stained cells) were counted in a blind manner using image-processing software (Image-J).

2.7. Western blot analysis

After treatment with different concentrations of COSs (0, 50, 100, 200 μM), ARPE-19 cells were incubated for 1 h. The cells were exposed to 2500 lx of blue light LED for 24 h. Then, the cells were washed with PBS, lysed in RIPA buffer (Sigma-Aldrich, St. Louis, MO, USA) containing 1% protease inhibitor cocktail and 1% phosphatase inhibitor cocktails 2 and 3 (Sigma-Aldrich, St. Louis, MO, USA), and harvested. Lysates were centrifuged at 12,000*g* for 15 min at 4 °C. Protein concentrations were measured using a BCA Protein Assay Kit with bovine serum albumin as the standard. Thereafter, an equal volume of protein sample and sample buffer were mixed, and the samples were boiled for