Blue light decreases oxidative stress defenses in an in vitro model of AMD

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Introduction

Blue light is an identified risk factor for age-related macular degeneration (AMD). Using a custom-made illumination system delivering 10 nm-wide illumination bands within the blue-green range, we recently showed that the narrow range 415-455 nm is the most toxic for A2E-loaded RPE cells (Arnault et al., 2013) and displayed high levels of ROS. To further understand the mechanisms involved in this phototoxicity, we investigated the photomodulation of major oxidative stress defenses in this blue-green range of the visible spectrum.

Material & Methods

AMD cellular model

Primary cultures of porcine retinal pigment epithelium cells (RPE) were incubated for 6 hours in culture medium without serum and containing 0 to 20 µM of A2E.

Light conditions

A2E-loaded cells were washed and exposed for 15 hours to 10 nm-wide illumination bands centered from 390 to 520 nm in 10 nm increments (+ one band centered at 630 nm). Control cells were maintained in darkness (D) during all the experiments. Light irradiances were normalized with respect to the natural sunlight reaching the retina after being filtered by the ocular structures (I0 = 1.5 mW/cm²).

Oxidative stress

ATP synthase (red) and cellular tight junctions (green) were immuno-labeled with antibody raised against ATP synthase b-subunit andZO-1, nuclei were stained with DAPI. Images were acquired on an inverted confocal microscope (Olympus). Mitochondrial membrane potential was measured with Mitotracker Membrane Potential Cytotoxicity kit (Enzo). mRNA expression levels were assessed by qRT-PCR and normalized to ribosomal 18S mRNA. Hydrogen peroxide was measured with ROS-Glo™ H2O2 Assay (Promega). Statistics: two-way ANOVA with repeated measures was used. Differences to 0 µM dark control (*) or 20 µM dark control (**) were considered significant for p<0.05 (*), p<0.01 (**), p<0.001 (***) or p<0.0001 (****).

1-Cellular model & Blue light illumination system

A. LED-based fibered cell illumination device
B. Five 16-well subdivisions were simultaneously exposed to five distinct illumination bands while the last subdivision remained in darkness.
C. Confocal imaging of A2E accumulation in RPE cells.
D. Irradiances at well-plate level proportional to daylight received onto the retina (mean ± s.e.m, n=4 to 6).

2-Mitochondria localization and shape

Under blue-violet light (430-440 nm) and in the presence of A2E, mitochondria were clustered around the nucleus and exhibited a globular shape. (Mean ± s.e.m, n=3)

3-Mitochondrial dysfunction

Mitochondrial membrane potential significantly decreased after blue-violet light exposure at 440 nm in A2E-loaded cells (mean ± s.e.m, n=3).

5-High levels of hydrogen peroxide

High levels of hydrogen peroxide were detected in the blue-violet spectrum after 15h light exposure in the presence of A2E in comparison to dark control and red light, especially between 415 – 455 nm. (mean ± s.e.m, n=4)

Low expression levels of major antioxidant enzymes were detected after blue light exposure in the presence of A2E

In A2E-loaded RPE cells exposed to blue-violet light (430-440 nm), mitochondria exhibited a peri-nuclear clustering and a globular shape.

Mitochondria membrane potential significantly decreased in A2E-loaded RPE cells exposed to blue-violet light demonstrated a mitochondrial dysfunction.

Mitochondria

4-Low expression levels of antioxidant defenses

Decreases of SOD2, catalase and GPX1 mRNA expression levels were observed after blue light exposure during 15 hours in A2E-loaded cells in comparison to non-treated controls or A2E-loaded cells exposed to red light (mean ± s.e.m, n=3).

Catalase

GPX1

SOD1

SOD2

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