Physiological Effects of Blue Light on C2C12 Myoblasts

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Introduction

Our daily lives render us under constant exposure to blue light, its main sources being not only the Sun but also the screens of our much-exploited electronics, including computers and smartphones. Blue light is the visible light of wavelengths between 400 to 500 nm. Other wavelengths, red and UV have been studied extensively[1,2]. Moreover, previous studies regarding blue light have focused on other cell types, such as keratinocytes, melanoma cells, and fibroblasts[3,4,5]. However, there is an insufficient amount of prior research that focuses on the consequences of blue light irradiation on skeletal muscles *in vitro*, especially under the light parameters mentioned below. Here in this study, we explore the effects of blue light irradiation on the physiology of C2C12 myoblasts, including cell viability, proliferation, wound healing, apoptosis, ROS levels, and DNA damage.

Material & Methods

<Cell culture>

Mouse skeletal muscle C2C12 cells were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS) at 37 ℃ in 5 % CO_2.

<Blue light parameters>

Cells were irradiated by a blue light-emitting diode (LED) lamp, which conditions were illuminance of 1000 lux, at wavelength of 470 nm, and power density of 2.7 W/m^2. Cells were plated overnight and irradiated for 0, 0.5, 1, or 2 h.

<Experiments>

1) Cell viability / cytotoxicity was determined by the MTT assay. Cells were plated in 96-well plates at different cell densities: 1.0 x 10^4, 5.0 x 10^3, 1.0 x 10^3, and were measured either immediately or 24 h after the irradiation.

2) Cell proliferation assay was conducted by taking microscopic images from Day 0 to Day 3, and total cell numbers from each day were counted using ImageJ software.

3) Wound healing assay was conducted by creating artificial scratches on cell monolayers using pipette tips. Microscopic images were taken immediately and 18 h after the irradiation, which differences were calculated using ImageJ in order to compare the migration areas.

4) Apoptotic cells were visualized as according to the kit instructions and quantified by ImageJ software. The assay was executed 1 h after the irradiation.

5) Intracellular ROS levels were measured by DCF fluorescence intensity, detected by a plate reader. Cells were treated by DCFDA solution as according to the kit instructions at a concentration of 10 μM and were incubated at 37 ℃ for 20 minutes to stain.

6) DNA damage was assessed by observing and quantifying fluorescence intensity of γ-H2AX, a known marker for DNA double strand breaks.

7) DNA was extracted by the Sodium Iodide (NaI) method as according to the kit instructions, which was then performed electrophoresis on a 1.0 % agarose gel.

Results

1) For the measurement taken immediately after the irradiation, cell viability didn't decrease. For measurement taken 24 h after the irradiation, cell viability decreased dose-dependently. Overall, blue light showed low to no effect on cell viability.

3) Blue light inhibited cell proliferation in a dose-dependent manner.

2) Blue light inhibited wound-healing migration.

4) No differences in fluorescence intensity were detected between the test groups; thus, blue light did not induce apoptosis.

5) Intracellular ROS levels were increased by blue light.

6) DNA damage was increased by blue light.

7) No distinct DNA ladders were detected as an effect of blue light irradiation.

Discussion

In summary, blue light irradiation was found to have inhibitory effects on some aspects of the C2C12 skeletal muscle cell physiology, such as cell growth and migration, and induced ROS generation and DNA damage. Results from the MTT and proliferation assays indicate that blue light is dose-dependently antiproliferative towards the cells. Furthermore, blue light was shown to be preventative of wound healing activities. However, in regard to ROS and DNA damage assays, further investigation is required to establish a better understanding of the damage mechanism.

References

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