Journal of Photochemistry and Photobiology B: Biology 142 (2015) 197–203

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Effect of blue light emitting diodes on melanoma cells: Involvement of apoptotic signaling

Phil-Sun Oh, Kyung Suk Na, Hyosook Hwang, Hwan-Seok Jeong, SeokTae Lim, Myung-Hee Sohn, Hwan-Jeong Jeong *

Department of Nuclear Medicine, Chonbuk National University Medical School and Hospital, Jeonju, Jeonbuk 561-712, Republic of Korea
Molecular Imaging & Therapeutic Medicine Research Center, Chonbuk National University Medical School and Hospital, Jeonju, Jeonbuk 561-712, Republic of Korea
Cyclotron Research Center, Chonbuk National University Medical School and Hospital, Jeonju, Jeonbuk 561-712, Republic of Korea
Institute for Medical Sciences, Chonbuk National University Medical School and Hospital, Jeonju, Jeonbuk 561-712, Republic of Korea
Biomedical Research Institute, Chonbuk National University Medical School and Hospital, Jeonju, Jeonbuk 561-712, Republic of Korea

ARTICLE INFO

Article history:
Received 30 July 2014
Received in revised form 24 November 2014
Accepted 4 December 2014
Available online 12 December 2014

ABSTRACT

The present study was undertaken to examine whether blue LED irradiation induces cellular apoptosis in B16-F10 cells and whether it blocks the early growth of melanoma cells in mice. Irradiation with blue LED was observed to reduce cell viability and to induce apoptotic cell death, as accompanied by exposure of phosphatidylserine on the plasma outside membrane and an accumulation of a sub-G1 population. Furthermore, the mitochondrial membrane potential increased, and mitochondria-related apoptotic proteins (cytochrome c, caspase 3, and PARP) were observed. In addition, the level of intracellular superoxide anion ($O_2^{\cdot-}$) gradually increased. Interestingly the phosphorylation of p53 increased at earlier times under blue LED irradiation, but reduced after exposure for a longer time. Additionally, the thickness of the mice footpad injected with B16-F10 cells decreased significantly until the 9th day of blue LED irradiation, indicating the inhibition of the early growth rate of the melanoma cells. Our data demonstrate that blue LED irradiation induces apoptotic cell death by activating the mitochondria-mediated pathway and reduces the early growth rate of melanoma cells. Further studies are needed to elucidate the precise mechanism of blue LED in melanoma cells.

1. Introduction

Melanoma is one of the most aggressive cancers, and it is resistant to conventional treatment-including chemotherapy, radiation, and immunotherapy- with an increasing incidence of mortality [1]. Melanoma develops from melanocytes in the skin and grows vertically from the basement membrane, and presence of tumor is often detected late after it has become sufficiently large [2]. Despite the development of targeted molecular therapies, most patients presenting with metastatic melanoma relapse after chemotherapy, probably as a result of the resistance of the cancer cells to the chemotherapeutic agents. Moreover surgical excision, a standard treatment at early stages of the disease, is ineffective in patients with metastatic progression due to rapid growth of the tumor after vascularization [2,3]. Therefore novel anti-cancer therapies are needed to treat melanoma.

Apoptosis or programmed cell death is involved in tissue homeostasis and tumorigenesis and is characterized by chromatin condensation and formation of apoptotic bodies. The induction of apoptosis in melanoma cells is important to therapeutic methods because apoptosis disrupts tumor cells without causing inflammatory process [4,5]. Mitochondria-mediated apoptosis, one of the intrinsic apoptotic pathways, is characterized by mitochondrial membrane permeabilization and the release of pro-apoptotic proteins, such as cytochrome c, from the intermembrane space to the cytosol. This, in turn, triggers caspase-3 activation which then leads to DNA fragmentation and cell death [6,7].

Light emitting diodes (LEDs) have been developed to replace traditional light bulbs with energy-efficient light sources. Recently, LEDs have become a new the preferred light source for phototherapy as a result of their lower cost and variability of wavelengths that range from the ultraviolet to the near-infrared region of the spectrum. In particular, wavelengths within the visible spectrum are currently being used clinically treat dermatitis, Alzheimer's disease, and muscle analgesia as well as to remove bacteria

* Corresponding author at: Molecular Imaging Therapeutic Medicine Research Center, Jeonbuk Regional Cyclotron Research Center & Biotracer Imaging Lab., Department of Nuclear Medicine, Chonbuk National University Medical School & Hospital, 634-18 GeumAm-dong Duckjin-gu Jeonju-si, Jeollabuk-do 561-803, Republic of Korea. Tel.: +82 63 250 1674; fax: +82 63 255 1172.
E-mail address: jayjeong@jbnu.ac.kr (H.-J. Jeong).

http://dx.doi.org/10.1016/j.jphotobiol.2014.12.006
1011-1344/

in vitro [8–10]. Zhang et al. used 650-nm red LEDs to suppress tumor growth by decreasing VEGF expression in HeLa cell implanted mice and by inducing apoptosis in cells [11]. More recently, Choi et al. reported that irradiation with 635-nm red LED has anti-inflammatory effects by inhibiting cytokine production and mediating MAPK signaling in human gingival fibroblasts [12].

Blue light sources with wavelengths of 400–500 nm have been primarily used in dental treatments to bleach colored teeth [13,14]. Since it only penetrates about 2 mm into the skin, blue light has not been widely applied in photodynamic therapy and has been restrictively used to treat hyperbilirubinaemia in infantile jaundice [15]. Recently, a study on irradiation found that blue light LEDs were more effective than quartz tungsten halogen lamp (QTH) in inhibiting cellular proliferation in human gingival fibroblast via generation of intracellular reactive oxygen species (ROS) [16]. Other studies have pointed out that blue LED can damage retinal pigment epithelium cells and induce in photoreceptors via the mitochondrial respiratory chain system [17]. Sparsa et al. [18] and Ohara et al. [19] suggested that blue light exerts a cytostatic effect, not a cytocidal action on B16 melanoma cells. In addition, the phototoxic and bactericidal effect of blue LED irradiation was reported to also depend on the wavelength [20]. However the underlying mechanism by which irradiation with blue LEDs interacts with its molecular targets and promotes cellular damage is unclear.

In the present study, we examined whether irradiation with blue LED has anti-proliferative and pro-apoptotic effects on melanoma cells. To determine effects of blue LED irradiation, we evaluated the intracellular superoxide anion ($O_2^{\cdot-}$) level, mitochondrial membrane potential, cell cycle arrest, and caspase activation in B16-F10 cells, and also the anti-proliferative activity in mice.

2. Materials and methods

2.1. Chemicals

RPMI 1640, fetal bovine serum (FBS), amphotericin B, and gentamicin were obtained from GIBCO BRL (Bethesda, MD). The protease and phosphatase inhibitor cocktails and all other chemicals were obtained from Sigma–Aldrich (St. Louis, MO). The cytochrome c (#4272), phospho-p53 (#9284), caspase-3 (#9662), cleaved caspase-3 (#9664) and PARP (#9542) antibodies were purchased from Cell Signaling (Beverley, MA), and β-actin (sc-47778) antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

2.2. Cell culture and LED irradiation

The B16-F10 melanoma cell line was used for the *in vitro* and *in vivo* experiments. Cells were obtained from the Korean Cell Line Bank (Seoul, Korea), and were grown in RPMI 1640 supplemented with 10% FBS, 10 μg/ml gentamicin and 0.25 μg/ml amphotericin B. Then the cells were maintained in 5% CO_2 in a 37 °C humidified incubator. We used a blue LED with 450 nm wavelength at a power of 15.6 mW/cm^2 for the *in vitro* experiments and 0.6 mW/cm^2 for the *in vivo* experiments (supplied by Dr. Hyunwoo Kim, Department of Physics, Chonbuk National University, Korea).

2.3. Growth inhibition assay

Cell growth was determined by measuring absorbance of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma–Aldrich) in living cells as described previously [21]. In brief, cells (5×10^4 cells/well) were seeded in 4-well plates (Nunc) and were irradiated with blue LED for the indicated time. Following exposure to blue LED, the MTT solution (10 μl: 5 mg/ml in PBS) was added to each well of the 4-well plates. The plates were further incubated for 4 h at 37 °C, and the medium was subsequently removed from the plates by pipetting, and 200 μl isopropyl alcohol were added to each well to solubilize the formazan crystals. The optical density at 570 nm was measured using a microplate reader (Molecular devices, USA).

2.4. Apoptosis

Cells (5×10^5 cells/well) were seeded in a 60-mm culture dish and were exposed to blue LED for the indicated time. After blue LED exposure, the cells were collected and washed with ice-cold PBS. Then the obtained cells were suspended in a 500 μl annexin V binding buffer containing 5 μl of annexin V-FITC and 10 μl of PI, and they were then incubated for 15 min at room temperature in the dark, according to the manufacturer's instructions. Fluorescence was measured on a BD LSR flow cytometer (Becton Dickinson, NY) and the data was analyzed with the Cell Quest software (Becton Dickinson, NY). The experiments were performed three times with similar results.

2.5. Sub-G1 cell analysis

The sub-G1 cells were determined via staining with PI (Ex/Em = 488 nm/617 nm; Sigma–Aldrich), which is a fluorescent biomolecule that can be used to stain DNA. In brief, cells (5×10^5 cells/well) were seeded in a 60-mm culture dish and were exposed to blue LED for the indicated time. The total cells, including the floating cells, were then washed with PBS and were fixed in 70% (v/v) ethanol. Cells were washed again with PBS, and were then incubated with PI (10 μg/ml) with simultaneous RNase treatment at 37 °C for 30 min. The cellular DNA content was then measured using a BD LSR flow cytometer.

2.6. Determination of intracellular superoxide anion ($O_2^{\cdot-}$)

Intracellular $O_2^{\cdot-}$ levels were detected by means of an oxidation-sensitive fluorescent probe dye, dihydroethidium (DHE, Ex/Em = 518 nm/605 nm) which is highly selective $O_2^{\cdot-}$. Briefly, cells (5×10^5 cells/well) were exposed to blue LED for the indicated time and were incubated with 10 μM DHE for 20 min. After incubation, fluorescence intensity was measured detected using a FACStar flow cytometer (Becton Dickinson). The $O_2^{\cdot-}$ levels were expressed as mean fluorescence intensity which was calculated by CellQuest software (Becton–Dickinson).

2.7. Mitochondrial transmembrane potential ($\Delta\Psi m$)

The mitochondrial membrane was monitored using Rhodamine-123 fluorescent dye (Ex/Em = 485 nm/535 nm; Sigma), a cell-permeable cationic dye, that preferentially enters into mitochondria due to the highly negative mitochondrial membrane potential ($\Delta\Psi m$). Depolarization of $\Delta\Psi m$ results in the loss of Rhodamine-123 from the mitochondria and in the decrease in intracellular fluorescence. Briefly, cells were exposed to blue LED for the indicated time and were incubated with Rhodamine-123 (0.1 μg/ml) at 37 °C for 30 min. The intensity of Rhodamine-123 staining was determined using a BD LSR flow cytometer.

2.8. Protein extraction and western blotting

Protein extracts were prepared as previously described [22]. Briefly, the cells were solubilized in RIPA lysis buffer containing a protease and phosphatase inhibitor cocktail solution. The supernatant was used as a whole cell lysate following centrifugation at 14,000 g for 15 min. To prepare the cytoplasmic and mitochondrial