Journal of Photochemistry and Photobiology B: Biology 117 (2012) 13–18

Contents lists available at SciVerse ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

The photocytotoxicity of different lights on mammalian cells in interior lighting system

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ARTICLE INFO

Article history:
Received 17 February 2012
Received in revised form 26 June 2012
Accepted 7 August 2012
Available online 23 August 2012

Keywords:
Photocytotoxicity
Incandescent light
LED
Mammalian cell
ROS
Lipid peroxidation

ABSTRACT

In the present paper, two light sources commonly used in interior lighting system: incandescent light and light emitting diode (LED) were chosen to evaluate their influences on three kinds of mammalian cells, together with UVA and UVB, and the mechanism of the photocytotoxicity was investigated in terms of intracellular ROS production, lipid peroxidation, SOD activity and GSH level assays. The results showed that LED and incandescent light both had some photocytotoxicities. In the interior lighting condition (100 lx–250 lx), the cytotoxicities of LED and incandescent lamp on RF/6A cells (rhesus retinal pigment epithelium cell line) were stronger than that on two fibroblast cell lines, while the cytotoxicity of UVA and UVB on HS68 cells (fibroblast cell line) was highest in the tests. The mechanism analysis revealed that the photocytotoxicities of LED and incandescent lamp were both caused by cell lipid peroxidation. LED and incandescent light could promote the production of ROS, raise lipid peroxidation level and lower the activity of the antioxidant key enzymes in mammalian cells, and finally cause a number of cells death. However, the negative function of LED was significantly smaller than incandescent light and ultraviolet in daily interior lighting condition. And the significantly lower photocytotoxicity of LED might be due to the less existence of ultraviolet. Therefore, LED is an efficient and relative safe light source in interior lighting system, which should be widely used instead of traditional light source.

1. Introduction

Human life is inseparable from the light, whether outdoors or indoors. However, constantly exposure to potentially harmful lights, like ultraviolet (UV), will make human skin liable to aging process [1,2]. UV irradiation has deleterious effects on human skin, including sunburn, immune suppression, cancer, and photo-aging [3]. UVB, in particular, is the most hazardous environmental carcinogen known with regard to human health through generation of reactive oxygen species (ROS) [4,5]. The ROS results in the subsequent activation of complex signaling pathways, followed by matrix metalloproteinases (MMPs) induction in skin cells and degradation or synthesis inhibition of collagenous extracellular matrix in connective tissues [6].

The light environment, where people live, includes sunlight and artificial. Currently, there are several man-made light sources used for interior lighting, such as incandescent light, fluorescent, light emitting diode (LED), etc. Incandescent light is the first electric light invented by T.A. Edison in 1879, which is culminating in the modern production methods after many improvements during more than a hundred years. It can emit a continuous spectrum, and most of the energy distributes in the visible spectral region. The color rendering of incandescent light is the best in all of light resource [7]. But because of low luminous efficiency, power consumption, etc., governments are introduced their incandescent phase-out plan. Compared with the incandescent light, LED is a new light source. LED is a semiconductor component, which can convert electrical energy into visible light solid-state. LED itself has many advantages, such as high luminous efficiency, long life, dimmable and so on. Because of the unique working principle, LED has the flexibility to produce many required spectra [8].

Do the incandescent light and LED, which people are daily expose to, have no harm for people? This question is closely related to people's health. But, to our best knowledge, there is no systematical study on their photocytotoxicities on mammalian cells in daily interior lighting condition. Therefore, in the present study, two light sources, commonly used in interior lighting system: incandescent light and LED, were chosen to evaluate their influences on three kinds of mammalian cells for the first time, together with UVA and UVB (EB-160C/12, Spectronics Corporation, USA).

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http://dx.doi.org/10.1016/j.jphotobiol.2012.08.007

Furthermore, the mechanism of the photocytotoxicity was investigated in terms of intracellular ROS production, lipid peroxidation, SOD activity and GSH level assays.

2. Materials and methods

2.1. Materials

Incandescent light (Edison 25W/CL/GLS/E27, product number: 70331) was purchased from General Electric Company, with 25-W power, 2800 K ± 500 K color temperature and 25 lm/W luminous efficiency. LED (MASTER LED spotLV MR16, product number: 929000172308) was purchased from Koninklijke Philips Electronics N.V., with 7-W power, 4000 K ± 500 K color temperature and 60 lm/W luminous efficiency. E Series UV Hand-Held Lamps (EA-160/12 and EB-160C/12) were purchased from Spectronics Corporation, USA. EA-160/12, equipped with one 6-W integrally filtered BLB tube, provided UVA condition; EB-160C/12, equipped with one 6-W tube and filter assembly, provided UVB condition.

Commercial kits used for determining lactate dehydrogenase (LDH) and malondialdehyde (MDA) were obtained from the Jiancheng Institute of Biotechnology (Nanjing, China). Other chemicals used in these experiments of analytical grade were obtained from commercial sources.

2.2. Cell treatment

RF/6A cells (rhesus retinal pigment epithelium cell line, ATCC® number: CRL-1780™), HS68 cells (normal human foreskin cell line, ATCC® number: CRL-1635™) and 3T3-L1 cells (mouse embryonic fibroblast-adipose like cell line, ATCC® Number: CL-173™) were obtained from American type culture collection (ATCC), USA. The cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% bovine serum (BS), 2 mM glutamine and 1% streptomycin/penicillin on 100 mm plastic culture dishes (BD Falcon, USA), at 37 °C in a humidified atmosphere containing 5% CO_2 and the medium was changed every 2–3 days. Cells were incubated up to about 24 h and grown to about 80% confluence before experiments.

Cells in culture dishes were washed and then covered with 10 mL of Hanks balanced salt solution (1.3 mM $CaCl_2$, 5.4 mM KCl, 0.4 mM KH_2PO_4, 0.5 mM $MgCl_2{\cdot}6H_2O$, 0.4 mM $MgSO_4{\cdot}7H_2O$, 136.7 mM NaCl, 4.2 mM $NaHCO_3$ and 0.3 mM $NaH_2PO_4{\cdot}H_2O$). All cells were irradiated with the different lights for 20 min and then cultured for 24 h. There are four kinds of light sources used in the experiment, including LED, incandescent light, UVA and UVB. The illuminations of LED, incandescent light and UVB were 250 lx, and that of UVA was 100 lx. The illumination was controlled by dimmer switch (No. 56101, Simon Electric Co. Ltd., China) and Testo 545 illuminometer (Testo AG, Germany).

2.3. Cell viability assay

Cells were seeded onto 24-well plates, 24 h prior to the experiment at a density of 5×10^4 well^{-1}. Cells were exposed to the light sources for 20 min. The photocytotoxicity was evaluated 24 h after light source exposure using the MTT assay whereby the tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is reduced by intracellular dehydrogenases of viable living cells leading to the formation of purple formazan crystals [9].

After 24 h, culture medium was removed and cells were incubated for 2 h at 37 °C with MTT salt solution (0.5 mg/mL) in PBS. The MTT solution was then removed and the crystals were dissolved in 400 mL DMSO. The optical density of each well was read at 550 nm using a microplate reader (Bio-Rad Model 680, USA). Cell viability is expressed as a percentage of live cells compared to unexposed control.

2.4. Lactate dehydrogenase (LDH) release assay

Photocytotoxicity induced by different light sources was also assessed by LDH leakage into the culture medium. Following exposure to different lights as described above for the cell viability assay, the cells were harvested and the LDH activity was assayed spectrophotometrically following the decrease in the absorbance of NADH at 340 nm by LDH assay kit.

2.5. Intracellular ROS assay

Cells were seeded onto 24-well plates, 24 h prior to the experiment at a density of 5×10^4 well^{-1}. Cells were irradiated with different light source in PBS as described above for the cell viability assay. For detection of photo-induced intracellular ROS formation, the ROS-index probe, carboxy-H_2DCFDA, was used. This compound readily diffuses across cell membranes, is hydrolyzed by intracellular esterases, and in the presence of ROS, is oxidized to dichlorofluorescein which is highly fluorescent and whose emission maximum can be monitored at 530 nm [10]. After light exposure, PBS was removed from cells and replaced with a solution of carboxy-H_2DCFDA (final concentration 5 mM) in PBS. Cells were incubated in this solution for 30 min at 37 °C before fluorescence measurements, which were carried out using a dual scanning microplate spectrofluorometer (Spectramax Gemini; Molecular Devices, Sunnyvale, CA, USA) with 480 nm excitation and 530 nm emission.

2.6. Measurement of intracellular malondialdehyde (MDA)

The thiobarbituric acid assay (TBARS) was used to detect lipid peroxidation [11]. After treatment, cells were washed with PBS and homogenized in 300 μL 0.1% Triton X-100 (PBS, pH 7.4) through sonication on ice for 10 s. After incubation at 4 °C for 10 min, the homogenates were centrifuged at 10,000 rpm for 10 min, and the supernatants were used for assay. MDA content was measured with the MDA kit.

2.7. Superoxide dismutase (SOD) activity assay

SOD activity was measured based on the extent inhibition of amino blue tetrazolium formazan formation in the mixture of nicotinamide adenine dinucleotide, phenazine methosulphate and nitroblue tetrazolium (NADH–PMS–NBT) [12]. Assay mixture contained 0.1 mL of cell lysate, 1.2 mL of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 mL of PMS (186 μM), 0.3 mL of NBT (300 μM) and 0.2 mL of NADH (750 μM). Reaction was started by addition of NADH. After incubation at 30 °C for 90 s, the reaction was stopped by addition of 0.1 mL of glacial acetic acid. Reaction mixture was stirred vigorously with 4.0 mL of *n*-butanol. Color intensity of the chromogen in butanol was measured spectrophotometrically at 560 nm.

2.8. Glutathione (GSH) level assay

The glutathione content of cell suspensions was determined by the DTNB–GSSG reductase recycling assay as described in Anderson (1985), with some modifications [13]. Briefly, 200 μL of cell suspension was added to 200 μL of 10% (w/v) 5-sulfosalicylic acid for protein precipitation and centrifuged 2 min at 12,000 rpm. Supernatant aliquots were taken out for measurement of total glutathione (GSx) following the DTNB oxidation at 415 nm and compared with a standard curve. The final concentrations of the