Pre-Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
49(5) 1–10

DOI: 10.1177/03000605211016623
journals.sagepub.com/home/imr
SAGE

Ultraviolet light-emitting diode irradiation induces reactive oxygen species production and mitochondrial membrane potential reduction in HL-60 cells

Dong Xie[1,*], Yun-Long Li[2,*], Gui-Fen Wang[3], Jian Jiang[1] and Li-Rong Sun[1]

Abstract

Objective: Ultraviolet light-emitting diode (UV LED) irradiation at 280 nm has been confirmed to induce apoptosis in cultured HL-60 cells, but the underlying mechanisms remain unclear. This study aimed to investigate the effects of 280 nm UV LED irradiation on reactive oxygen species (ROS) production and mitochondrial membrane potential (MMP) in HL-60 cells.

Methods: HL-60 cells were irradiated with 0, 8, 15, or 30 J/m^2 of 280 nm UV LED and incubated for 2 hours. The intracellular ROS levels were assessed using the fluorescent probe 2′-7′-dichlorodihydrofluorescein diacetate (DCFH-DA) and a fluorescence plate reader. MMP was determined by flow cytometry using 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazol-carbocyanine iodide (JC-1) staining. The apoptosis-related proteins Bax and Bcl-2 were evaluated by western blot.

Results: UV LED irradiation at 280 nm induced a dose-dependent increase in ROS production and loss of MMP, and it activated apoptosis at irradiation doses of 8 to 30 J/m^2. These results were consistent with a previous apoptosis study from the authors' group.

Conclusion: Enhanced ROS production and mitochondrial depolarization are two distinct but interacting events, and both are involved in UV LED-induced apoptosis in HL-60 cells.

[1]Department of Pediatric Hematology, The Affiliated Hospital of Qingdao University, Qingdao, Shandong, China
[2]Department of Pediatrics, People's Hospital of Rizhao, Rizhao, Shandong, China
[3]Department of Obstetrics and Gynecology, Maternal and Child Care Service Centre of Zhaoyuan, Zhaoyuan, Shandong, China

*These authors contributed equally to this work.

Corresponding author:
Li-Rong Sun, Department of Pediatric Hematology, The Affiliated Hospital of Qingdao University, No. 16 Jiangsu Road, Qingdao, Shandong 266000, China.
Email: sunlr_pediatrics@sina.com

Keywords
Ultraviolet radiation, light-emitting diode, apoptosis, reactive oxygen species, mitochondrial membrane potential, mitochondrial depolarization

Date received: 5 November 2020; accepted: 19 April 2021

Introduction

Ultraviolet (UV) radiation induces apoptosis.[1] The biological effects of UV light are principally attributed to direct photochemical reactions within DNA or indirect oxidative stress from reactive oxygen species (ROS).[2,3] Three important, but not mutually exclusive, events (DNA damage, death receptor activation, and ROS formation) can be directly induced by UV radiation and independently contribute to apoptosis.[4,5] These events can initiate various signaling pathways that result in tumor suppressor gene *p53* phosphorylation, mitochondrial membrane potential (MMP) dissipation, and caspase cascade activation.[4,5]

Given their biological effects, traditional UV lamps have been widely used for disinfection and dermatosis treatment for decades.[6,7] However, owing to mercury toxicity and high energy consumption, alternative UV sources are being sought.[8] Recently, UV light-emitting diodes (UV LEDs) have emerged as a new UV radiation source. LED is a semiconductor device that uses semiconducting materials to create a p–n junction between two semiconductor materials, which emits light.[9] The emitting wavelengths of LEDs are determined by different semiconducting materials, including gallium nitride (GaN), aluminum nitride (AlN), and aluminum gallium nitride (AlGaN).[9] The new UV LEDs are recommended as a substitute for traditional mercury lamps in numerous applications such as UV curing, decontamination, and phototherapy because they have numerous advantages, including non-toxicity, greater energy efficiency, greater operational flexibility, a faster start-up time, and a longer lifetime. Moreover, they can emit narrower and more desirable wavelengths than traditional UV lamps.[9–11]

The substantial differences between the two traditional UV lamps and UV LEDs make it unlikely that UV mercury lamp protocols for cell apoptosis could be directly applicable to UV LEDs in *in vitro* studies. It has been reported that 280 nm UV LED irradiation has a direct inhibitory effect on *Trichophyton rubrum* spores *in vitro*.[12] Our previous study showed that 280 nm AlGaN-based UV LED irradiation (8–30 J/m^2) inhibits *Bcl-2* mRNA expression and induces dose-dependent apoptosis and cell cycle arrest in HL-60 cells.[13]

This study aimed to examine the effects of UV LED irradiation on intracellular ROS levels and MMP in cultured HL-60 cells. The results could help to provide new insights into cellular responses to UV LED irradiation.

Materials and methods

Cell culture and irradiation

All protocols in this study were reviewed by the Review Board of Affiliated Hospital of Qingdao University, and the study received an exemption because the study did not involve animals or humans (Review Board number AHQU20161013). HL-60 cells were obtained from the American Type Culture

Collection (ATCC, Manassas, VA, USA) and cultured in Iscove's modified Dulbecco's medium (IMDM, SH30228.01B, Hyclone, Logan, UT, USA) with 10% fetal bovine serum (FBS, SH30088.03, Hyclone) in a 37°C incubator (Thermo Fisher Scientific Inc., Waltham, MA, USA) with a humidified atmosphere of 5% CO_2. Cells that were in the exponential growth phase were plated in 24-well plates at 1×10^6 cells/well in triplicate. When they grew to confluence, the cells were subjected to UV LED irradiation (Qingdao Ziyuan Photoelectronic Co., Ltd., Qingdao, China) at 0, 8, 15, or 30 J/m^2 and incubated for 2 hours.

Cell morphology

Cell morphology was observed using an inverted microscope (CKX41; Olympus Corporation, Tokyo, Japan).

Measurement of ROS

Intracellular ROS levels were assessed by epifluorescence using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA, S0033, Beyotime Institute of Biotechnology, Haimen, China). DCFH-DA is a nonpolar membrane-permeable probe that can be hydrolyzed by intracellular esterase to 2′,7′-dichlorodihydrofluorescein (DCFH), which can be oxidated to highly fluorescent 2′,7′-dichlorofluorescein (DCF) in the presence of ROS and peroxides.[14] After irradiation treatment, HL-60 cells were harvested, loaded with 10 μM DCFH-DA at 1×10^7 cells/mL, and incubated for 20 minutes in the dark at 37°C. The cells were washed with serum-free medium and visualized under a fluorescence microscope (DMI 3000 B; Leica Microsystems, Wetzlar, Germany). The fluorescence intensity was determined using a fluorescence plate reader (Millipore, Billerica, MA, USA) at 485 nm excitation and 535 nm emission. The arbitrary DCF fluorescence units were normalized as 100% in the control condition (0 J/m^2). For fluorescence microscopy (DMI 3000 B; Leica Microsystems) validation, 100 cells were examined, and the cells that showed florescent light were counted.

Assessment of MMP changes

MMP was determined by flow cytometry (FC 500 MPL; Beckman Coulter Inc., Fullerton, CA, USA) using 5,5′,6,6′-tetrachloro-1,1′,3, 3′-tetraethylbenzimidazolcarbocyanine iodide (JC-1) staining. JC-1 (C2006-1, Beyotime Institute of Biotechnology) is a lipophilic cationic dye that can selectively enter into mitochondria; its color reversibly changes with mitochondrial membrane depolarization. In normal cells with a high MMP, JC-1 spontaneously forms complexes (known as J-aggregates) and exhibits intense red fluorescence. In unhealthy cells with a low MMP, JC-1 remains in its monomeric form and exhibits green fluorescence.[14] HL-60 cells were harvested and incubated with JC-1 at 37°C for 20 minutes at 1×10^6 cells/mL. The cells were washed with JC-1 dye buffer (C2006-3, Beyotime Institute of Biotechnology) and analyzed using the FlowJo flow cytometry analysis software (FlowJo LLC, Ashland, OR, USA) at 525 nm excitation and 590 nm emission.

Analysis of Bax and Bcl-2 protein expression

HL-60 cells were irradiated with different doses of 280-nm UV LED and cultured for 2 hours. The cells were harvested and lysed with RIPA lysis buffer (P0013C, Beyotime Institute of Biotechnology). The proteins (15 μg) were separated on a 12% SDS-PAGE gel (P0012A, Beyotime Institute of Biotechnology) and transferred onto polyvinylidene difluoride membranes

(PVDF, ISEQ00010, Millipore). The membranes were incubated with primary antibodies against glyceraldehyde 3-phosphate dehydrogenase (GAPDH; ab128915, Abcam, Cambridge, UK), Bax (ab182733, Abcam), and Bcl-2 (ab32124, Abcam) at 4°C overnight. The membranes were then incubated with the corresponding secondary antibodies (ab205718, Abcam) at room temperature for 1 hour. The signals were visualized using chemiluminescence (Bio-Rad Laboratories, Hercules, CA, USA). ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used to analyze the data.

Determination of DNA damage in human lymphocytes

Please see the detailed information in the Supplementary Materials.

Statistical analysis

The results were analyzed using SPSS 22.0 (IBM Corp., Armonk, NY, USA). The data were expressed as the mean ± standard deviation and analyzed using one-way analysis of variance (ANOVA) followed by the Bonferroni correction for multiple pairwise comparisons. A P value <0.05 was considered to be statistically significant.

Results

Cell morphology

The non-irradiated cells were spherical and arranged in an orderly manner, while their irradiated counterparts had an irregular morphology and were disordered. The transmittance tended to decrease with an increase in the irradiation doses (Figure 1).

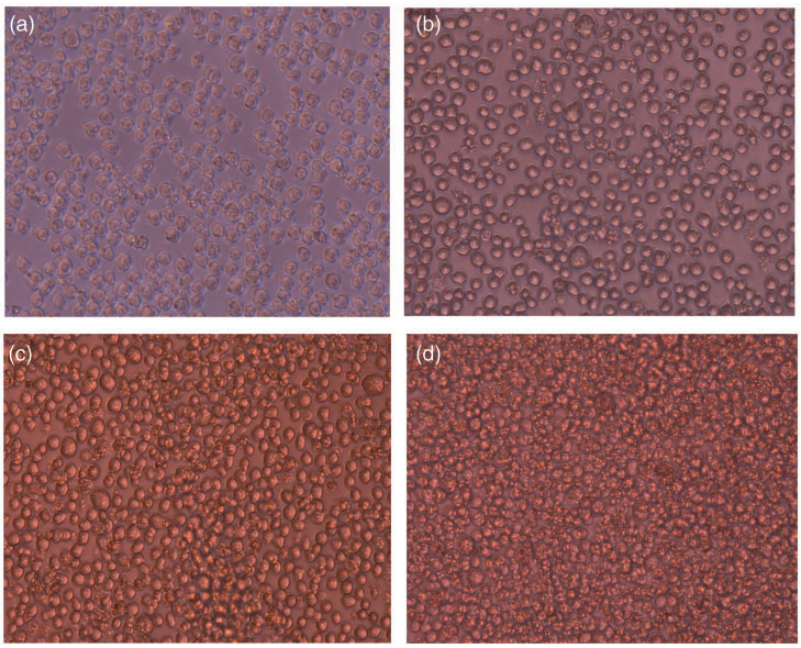

Figure 1. Morphological characteristics of HL-60 cells. HL-60 cells were exposed to UV LED irradiation at doses of (a) 0, (b) 8, (c) 15, or (d) 30 J/m^2 and incubated for 2 hours. The cells were observed under an inverted microscope. Magnification, 200×.
UV LED, ultraviolet light-emitting diode.

UV LED irradiation increased the amount of ROS in HL-60 cells

Intracellular ROS levels were determined using a fluorescence plate reader and confirmed by fluorescence microscopy. A dose-dependent increase in DCF fluorescence was observed with increasing irradiation doses, indicating that UV LED irradiation (8–30 J/m^2) could induce ROS production in HL-60 cells ($P<0.05$ for all doses; Figure 2).

UV LED irradiation induced loss of MMP in HL-60 cells

Changes in MMP were determined by detecting the red fluorescence intensity. Upon exposure to UV LED irradiation, the percentage of red fluorescent cells decreased with the increase in irradiation doses from 93.8% in the 0 J/m^2 group to 8.02% in the 30 J/m^2 group ($P<0.05$ for all comparisons), suggesting that UV LED irradiation (8–30 J/m^2) could lower the MMP in a dose-dependent manner (Figure 3).

UV LED irradiation upregulated Bax protein and downregulated Bcl-2 protein levels in HL-60 cells

Western blot results showed that UV LED irradiation at 8 to 30 J/m^2 induced a dose-dependent increase in Bax protein and a decrease in Bcl-2 protein levels, which is consistent with the previous apoptosis and MMP studies by the authors' group.[13] The changes in pro-apoptotic Bax and anti-apoptotic Bcl-2 might be involved in the loss of MMP and apoptosis ($P<0.05$ for multiple pairwise comparisons; Figure 4).

UV LED irradiation induced DNA damage in human lymphocytes

Please see the detailed information in the Supplementary Materials and Figure S1.

Antioxidants antagonized DNA damage of lymphocytes induced by UV LED irradiation

Please see the detailed information in the Supplementary Materials and Figure S2.

Discussion

ROS is induced by UV irradiation via two primary pathways: direct and indirect. The direct pathway involves absorption of energized UV photons by cellular photosensitizers, causing an initial ROS burst through energy transfer from excited photosensitizers to molecular oxygen.[2,3] The indirect pathway is termed mitochondrial ROS-induced ROS release (RIRR), in which the initial ROS burst reaches threshold levels for opening the mitochondrial membrane channels, resulting in MMP dissipation and mitochondrial ROS production by the electron transfer chain. Generated ROS are released into the cytosol and serve as a "second messenger" to activate RIRR in adjacent mitochondria, inducing a positive-feedback loop for enhanced ROS production.[15]

When the amount of ROS overwhelms the antioxidant capacity of the cell, oxidative damage to cellular components (such as DNA, lipids, and proteins) ensues, perturbing cellular functions and ultimately leading to apoptosis.[5,16] ROS can directly trigger cytochrome c release, thereby independently contributing to UV-induced apoptosis.[4,5] In addition, ROS, as secondary messengers, mediate the activation of apoptosis-related signaling cascades such as mitogen-activated protein kinases (MAPKs) and nuclear factor κB (NF-κB).[17,18] This study found that 280 nm UV LED irradiation induced dose-dependent ROS production at 8 to 30 J/m^2, which was consistent with our previous findings that apoptosis could be induced under the same UV LED regimen. This indicates that UV LED-induced apoptosis in HL-60 cells is potentially

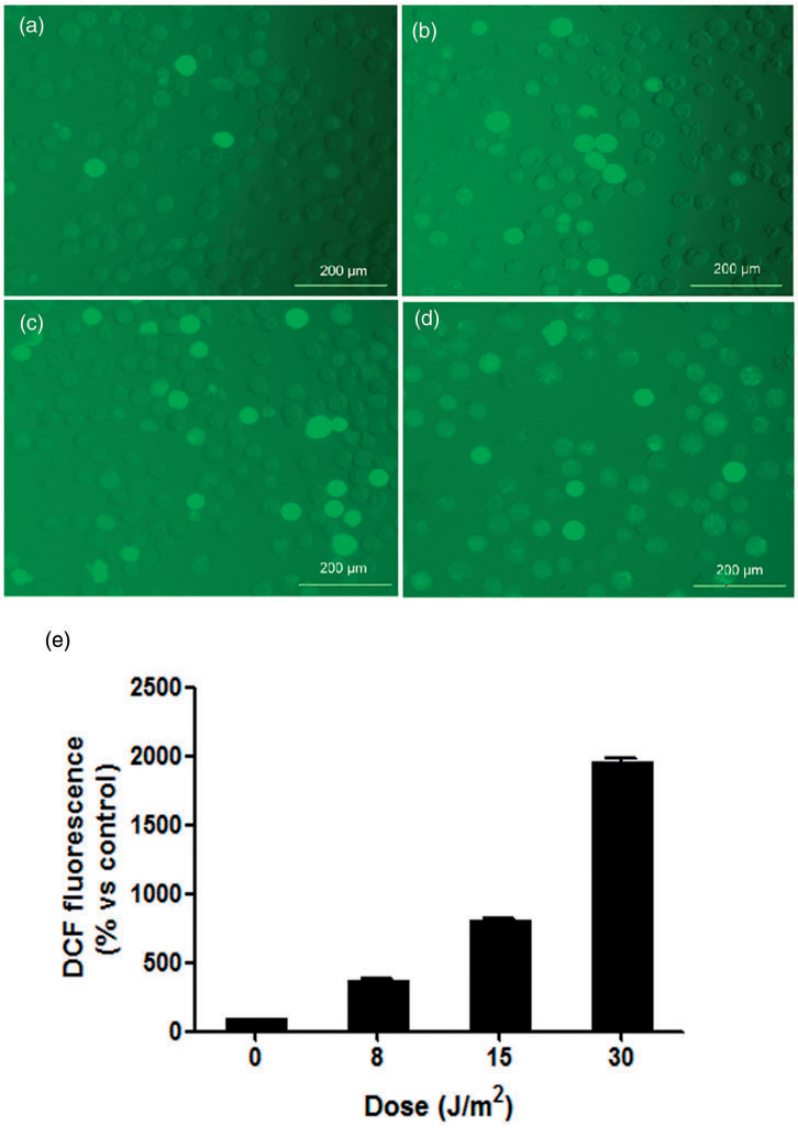

Figure 2. UV LED irradiation induced ROS production in HL-60 cells. Intracellular ROS levels were positively correlated with the percentage of fluorescence-activated cells that was determined by epifluorescence using a DCFH-DA probe. The fluorescent cells were observed under a fluorescence microscope after exposure to UV LED irradiation at doses of (a) 0, (b) 8, (c) 15, or (d) 30 J/m^2. Magnification, 200×. (e) UV LED irradiation induced a dose-dependent increase in the DCF fluorescence intensity. $P<0.05$ for multiple pairwise comparisons.

UV LED, ultraviolet light-emitting diode; ROS, reactive oxygen species; DCFH-DA, 2′,7′-dichlorodihydrofluorescein diacetate; DCF, 2′,7′-dichlorofluorescein.

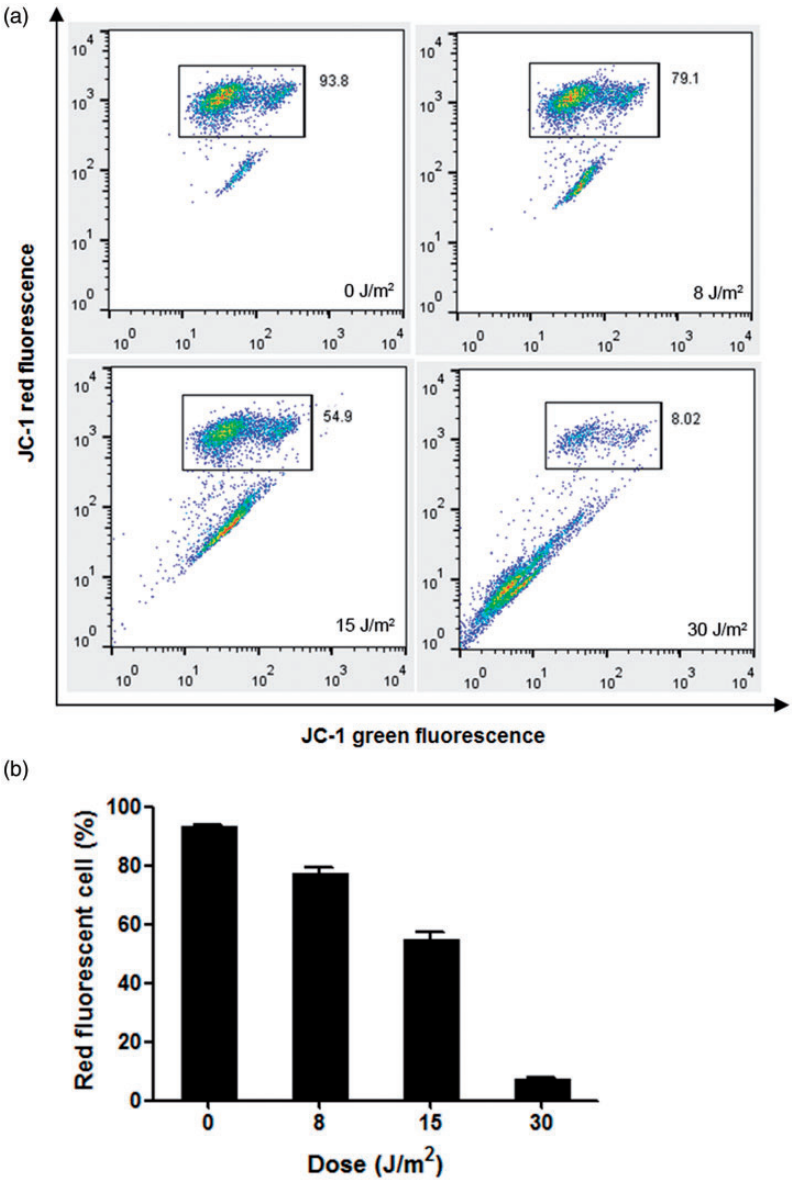

Figure 3. UV LED irradiation induced MMP reduction in HL-60 cells. (a) MMP was detected by flow cytometry using JC-1 staining after the cells were subjected to UV LED irradiation at doses of 0, 8, 15, or 30 J/m^2, which was negatively correlated with the red fluorescence intensity. (b) UV LED irradiation induced a dose-dependent decrease in the percentage of red fluorescent cells. $P<0.05$ for multiple pairwise comparisons.

UV LED, ultraviolet light-emitting diode; MMP, mitochondrial membrane potential; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazol-carbocyanine iodide.

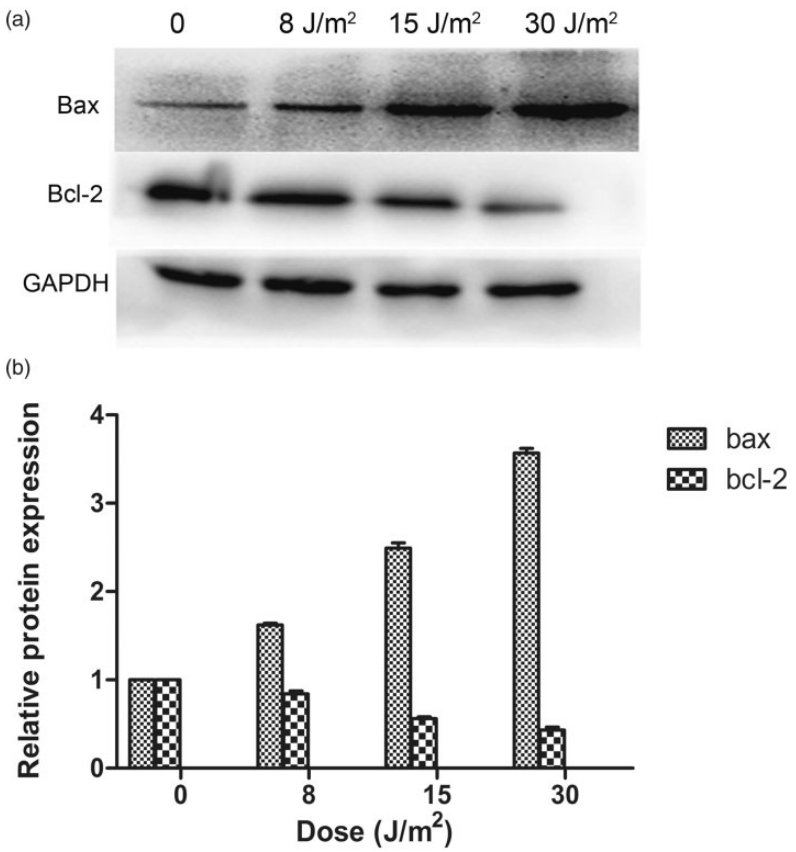

Figure 4. UV LED irradiation upregulated Bax protein and downregulated Bcl-2 protein levels in HL-60 cells. (a) The cells were irradiated with UV LED at 0, 8, 15, or 30 J/m^2. Western blot analysis was performed to detect Bax and Bcl-2 expression. GAPDH served as a loading control. (b) The relative Bax protein expression was upregulated as the UV LED dose increased, while the relative Bcl-2 protein expression was downregulated in a dose-dependent manner. $P<0.05$ for multiple pairwise comparisons.
UV LED, ultraviolet light-emitting diode; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

associated with enhanced ROS production. UV light damages DNA directly, and the ROS that are generated following UV irradiation also damage DNA.[4,5] In the present study, UV LED damaged lymphocyte DNA, and antioxidants partially prevented this damage, which suggests that there are direct and indirect effects of UV LED on DNA (Figures S1, S2).

MMP is essential for cellular metabolism and mitochondrial functions.[19] Recent studies have indicated that MMP dissipation is the most predominant event during the early stages of the intrinsic apoptotic pathway. Multiple apoptotic signals converge on the mitochondria, such as increased ROS production and *p53* and MAPK activation, leading to the translocation and oligomerization of the pro-apoptotic Bax/Bak proteins, which result in the loss of MMP and subsequent release of apoptogenic proteins (e.g., cytochrome c, apoptosis-inducing factors, and Smac/Diablo) into the cytoplasm. The resulting proteolytic processes trigger activation of the caspase cascade, which ultimately leads to apoptosis.[20,21] In the present

study, a dose-dependent decrease in MMP was observed after 8 to 30 J/m^2 UV LED irradiation, which was in accordance with the results of the previous apoptosis study by the authors' group.[13] This suggests that MMP dissipation was involved in UV LED-mediated apoptosis of HL-60 cells. In addition, the changes in pro-apoptotic Bax and anti-apoptotic Bcl-2 support activation of apoptosis with higher UV LED doses.

This study has some limitations. Only two types of eukaryotic cells were investigated. Future studies should examine bacteria, fungi, and viruses. Apoptosis is a complex event, and only a few components of apoptosis were examined. In addition, a standard mercury UV lamp was not used in this study. Future studies could compare the effects of the two types of UV light in parallel.

Taken together, ROS production and MMP reduction are two distinct but interacting events, both of which participate in UV LED-mediated apoptosis. The elucidation of additional molecular mechanisms underlying apoptosis and the interplay between the different pathways will increase our understanding of how UV LED irradiation exerts its apoptotic effects. This might facilitate the development of strategies for killing various types of cells.

Author contributions

Dong Xie and Lirong Sun conceived and designed the study. Guifen Wang conducted the cell culture and western blot experiments. Dong Xie and Yunlong Li assessed ROS and MMP. Jian Jiang was the data manager for the study. Dong Xie drafted the manuscript. Lirong Sun performed a critical revision of the manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

Availability of data and materials

The datasets that were used and analyzed during the current study are available from the corresponding author upon reasonable request.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

ORCID iD

Li-Rong Sun https://orcid.org/0000-0003-2843-4719

Supplemental material

Supplementary material for this article is available online.

References

1. Salucci S, Burattini S, Battistelli M, et al. Ultraviolet B (UVB) irradiation-induced apoptosis in various cell lineages in vitro. *Int J Mol Sci* 2012; 14: 532–546. DOI: 10.3390/ijms14010532.
2. Brand RM, Wipf P, Durham A, et al. Targeting mitochondrial oxidative stress to mitigate UV-induced skin damage. *Front Pharmacol* 2018; 9: 920. DOI: 10.3389/fphar.2018.00920.
3. Panich U, Sittithumcharee G, Rathviboon N, et al. Ultraviolet radiation-induced skin aging: the role of DNA damage and oxidative stress in epidermal stem cell damage mediated skin aging. *Stem Cells Int* 2016; 2016: 7370642. DOI: 10.1155/2016/7370642.
4. Lee CH, Wu SB, Hong CH, et al. Molecular mechanisms of UV-induced apoptosis and its effects on skin residential cells: the implication in UV-based phototherapy. *Int J Mol Sci* 2013; 14: 6414–6435. DOI: 10.3390/ijms14036414.
5. Redza-Dutordoir M and Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim Biophys Acta* 2016; 1863: 2977–2992. DOI: 10.1016/j.bbamcr.2016.09.012.
6. Gupta A, Avci P, Dai T, et al. Ultraviolet radiation in wound care: sterilization and stimulation. *Adv Wound Care (New*

Rochelle) 2013; 2: 422–437. DOI: 10.1089/wound.2012.0366.
7. Ahmed I, Fang Y, Lu M, et al. Recent patents on light-based anti-infective approaches. *Recent Pat Antiinfect Drug Discov* 2018; 13: 70–88. DOI:10.2174/1872213X11666171108104104.
8. Sholtes KA, Lowe K, Walters GW, et al. Comparison of ultraviolet light-emitting diodes and low-pressure mercury-arc lamps for disinfection of water. *Environ Technol* 2016; 37: 2183–2188. DOI: 10.1080/09593330.2016.1144798.
9. Song K, Mohseni M and Taghipour F. Application of ultraviolet light-emitting diodes (UV-LEDs) for water disinfection: a review. *Water Res* 2016; 94: 341–349. DOI: 10.1016/j.watres.2016.03.003.
10. Kwon TR, Oh CT, Choi EJ, et al. Ultraviolet light-emitting-diode irradiation inhibits TNF-α and IFN-γ-induced expression of ICAM-1 and STAT1 phosphorylation in human keratinocytes. *Lasers Surg Med* 2015; 47: 824–832.
11. Ibrahim MA, MacAdam J, Autin O, et al. Evaluating the impact of LED bulb development on the economic viability of ultraviolet technology for disinfection. *Environ Technol* 2014; 35: 400–406. DOI: 10.1080/09593330.2013.829858.
12. Cronin LJ, Mildren RP, Moffitt M, et al. An investigation into the inhibitory effect of ultraviolet radiation on Trichophyton rubrum. *Lasers Med Sci* 2014; 29: 157–163. DOI: 10.1007/s10103-013-1287-4.
13. Xie D, Sun Y, Wang L, et al. Ultraviolet light-emitting diode irradiation-induced cell death in HL-60 human leukemia cells in vitro. *Mol Med Rep* 2016; 13: 2506–2510. DOI: 10.3892/mmr.2016.4812.
14. Yao C, Jiang J, Tu Y, et al. β-element reverses the drug resistance of A549/DDP lung cancer cells by activating intracellular redox system, decreasing mitochondrial membrane potential and P-glycoprotein expression, and inducing apoptosis. *Thorac Cancer* 2014; 5: 304–312. DOI: 10.1111/1759-7714.12093.
15. Zorov DB, Juhaszova M and Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev* 2014; 94: 909–950. DOI: 10.1152/physrev.00026.2013.
16. Dhumrongvaraporn A and Chanvorachote P. Kinetics of ultraviolet B irradiation-mediated reactive oxygen species generation in human keratinocytes. *J Cosmet Sci* 2013; 64: 207–217.
17. Feng R, Han J, Ziegler J, et al. Apaf-1 deficiency confers resistance to ultraviolet-induced apoptosis in mouse embryonic fibroblasts by disrupting reactive oxygen species amplification production and mitochondrial pathway. *Free Radic Biol Med* 2012; 52: 889–897. DOI: 10.1016/j.freeradbiomed.2011.11.028.
18. Acharya A, Das I, Chandhok D, et al. Redox regulation in cancer: a double-edged sword with therapeutic potential. *Oxid Med Cell Longev* 2010; 3: 23–34. DOI: 10.4161/oxim.3.1.10095.
19. Zorova LD, Popkov VA, Plotnikov EY, et al. Mitochondrial membrane potential. *Anal Biochem* 2018; 552: 50–59. DOI: 10.1016/j.ab.2017.07.009.
20. Westphal D, Kluck RM and Dewson G. Building blocks of the apoptotic pore: how Bax and Bak are activated and oligomerize during apoptosis. *Cell Death Differ* 2014; 21: 196–205. DOI: 10.1038/cdd.2013.139.
21. Gillissen B, Richter A, Richter A, et al. Bax/Bak-independent mitochondrial depolarization and reactive oxygen species induction by sorafenib overcome resistance to apoptosis in renal cell carcinoma. *J Biol Chem* 2017; 292: 6478–6492. DOI: 10.1074/jbc.M116.754184.