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Research Article

Photobiomodulation for Cobalt Chloride-Induced Hypoxic Damage of RF/6A Cells by 670 nm Light-Emitting Diode Irradiation

Shuang Li,[1] Qiang-Li Wang,[2] Xin Chen,[1] and Xian-qiang Mi[1,3,4]

[1] *College of Medical Device and Food Engineering, University of Shanghai for Science and Technology, Shanghai 200093, China*
[2] *Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*
[3] *Key Laboratory of System Biology, Chinese Academy of Sciences, Shanghai 201210, China*
[4] *Laboratory of System Biology, Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai 201210, China*

Correspondence should be addressed to Xian-qiang Mi; mixq@sari.ac.cn

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Objective. The goal of this study was to investigate the therapeutic efficacy of 670 nm light-emitting diode (LED) irradiation on the diabetic retinopathy (DR) using hypoxic rhesus monkey choroid-retinal (RF/6A) cells as the model system. *Background Data*. Treatment with light in the spectrum from red to near-infrared region has beneficial effect on tissue injury and 670 nm LED is currently under clinical investigation for retinoprotective therapy. *Methods*. Studies were conducted in the cultured cells under hypoxia treated by cobalt chloride ($CoCl_2$). After irradiation by 670 nm LED with different power densities, cell viability, cytochrome C oxidase activity, and ATP concentration were measured. *Results*. The irradiation of 670 nm LED significantly improved cell viability, cytochrome C oxidase activity, and ATP concentration in the hypoxia RF/6A cells. *Conclusion*. 670 nm LED irradiation could recover the hypoxia damage caused by $CoCl_2$. Photobiomodulation of 670 nm LED plays a potential role for the treatment of diabetic retinopathy.

1. Introduction

Diabetic retinopathy (DR) is one of the most serious diabetic microvascular complications affecting large number of patients. DR causes retinal capillary damage [1] and ultimately leads to blindness. Despite the wide-spread applications of retinal laser photocoagulation in the treatment of DR, there are serious side effects as the treatment itself is a pathological process. New treatment methods for DR are being actively explored and photobiomodulation for DR is one of the most promising therapies.

Photobiomodulation has been demonstrated to be able to modulate various biological processes in cell culture and animal models [2] including accelerating wound healing, improving mitochondrial function, and attenuating degeneration in the injured optic nerve [3–6]. Low level laser or light emitting diodes (LEDs) are the most common light sources for photobiomodulation. Compared with laser, LED shows more promising future as it has less heat production and toxic side effects.

Previous studies have provided evidence for the therapeutic benefit of LED treatment at 670 nm in improvement of oxidative metabolism of mitochondria *in vitro* and functional recovery of retinal after acute injury by the mitochondrial toxin *in vivo* [4, 7]. It indicates that cytochrome C oxidase which plays an important role in generating ATP is a primary photoreceptor of light in the red to near-IR region of the spectrum [8–10].

Here, we demonstrate that the protective effect of 670 nm LED on RF/6A cells results from the stimulation of cellular events associated with the enhancement of cytochrome C oxidase activity, further improves oxidative metabolism of mitochondria, and provides protection against hypoxic damage. We employed cytochrome c oxidase activity and ATP content

as the sensitive indicators after hypoxic caused by $CoCl_2$ and demonstrated the efficacy of 670 nm LED treatment delivered one time per day. We proposed that photobiomodulation of LED represents an innovative and noninvasive therapeutic approach for the treatment of diabetic retinopathy.

2. Materials and Methods

2.1. Materials. LED device was obtained from Shenzhen Lamplic Technology Company Limited. The retinal vascular endothelial cell line RF/6A was obtained from Cell Bank, Chinese Academy of Sciences. MTT and $CoCl_2$ were obtained from Sigma-Aldrich Corporation (mainland). Mitochondria cytochrome C oxidase activity kit was purchased from Genmed Scientifics Inc., USA. Mitochondria isolation kit, BCA protein concentration determination kit, and ATP Assay Kit were purchased from Shanghai Beyotime Institute of Biotechnology.

2.2. Cell Cultures. RF/6A cells were cultured in DMEM containing 10% fetal bovine serum (1% streptomycin and penicillin) under 5% CO2, 37∘C, and passaged per 2-3 d.

2.3. Hypoxic Model System Induced by $CoCl_2$. Hypoxic model system was produced by the rhesus monkey choroid-retinal (RF/6A) cells incubated with $CoCl_2$ for 24 h. Controls group was the RF/6A cells without cobalt chloride.

2.4. LED Treatment. RF/6A cells with $CoCl_2$ treatment were irradiated for 71 seconds in the dark with 670 nm LED once a day for 3 days.

2.5. Cell Viability. Cell viability was determined by MTT assay. Cells in good conditions was transferred to cell suspension and then added to 96-well plates at 5×10^4/mL (100 μL each well). MTT solution (5 mg/m1, 20 μL) was added to each well and then cultured in CO_2 incubator for 4 h. After the medium was removed, 200 μL DMSO was added and shaked 10 min. OD value was obtained by ELISA Reader.

2.6. Cytochrome C Oxidase Assays. Cytochrome C oxidase activity was determined by the change of light absorption value with colorimetry by spectrophotometer at 550 nm. 30 μL mitochondrial lysate was added to split mitochondria. Guided by Mitochondria Cytochrome C Oxidase Activity Kit Introductions provided by manufacturer, samples were prepared. After buffer and samples were added, OD values were measured by spectrophotometer. Cytochrome C oxidase activity of samples was calculated and normalized based on OD values and protein concentration.

2.7. ATP Assay Kit. The assay is based on the firefly luciferase-catalyzed oxidation of D-luciferin in the presence of ATP and oxygen, whereby the amount of ATP is quantified by the amount of light (hv) produce. Cultured cells were rinsed with cold phosphate-buffered saline, harvested from the cover slips by means of a cell scraper, and then centrifuged at 4°C, 12000 g for 8 min with supernatant left. 100 μL ATP testing reagent was added and incubated at room temperature for 3–5 min to exhaust the ATP in background. Then mixed with the luciferase ATP assay and assayed with a luminometer.

2.8. Statistical Analysis. All values are expressed as means ± SEM. A one way ANOVA was used in SPSS13.0 to determine whether any significant differences existed among groups. In all cases, the minimum level of significance was taken as $P < 0.05$.

3. Result

3.1. The Determinant of the Optimal $CoCl_2$ Concentration. The RF/6A model cells were divided into control group and hypoxia groups induced by different concentration of $CoCl_2$ (100, 200, 300, 400, and 500 μmol/L). Cell viability was measured by MTT method. It showed that cell viability decreased in a power density dependent manner (Figure 1). There is significant difference among cell viability of 200 μmol/L, 300 μmol/L, 400 μmol/L, and 500 μmol/L $CoCl_2$ damaged groups and control group. Here, 200 μmol/L $CoCl_2$ was chosen for the following up experiments.

3.2. The Determinant of the Optimal LED Power Density. It demonstrated that, compared with the control group, the cell viability of both the RF/6A model cell damaged by 200 μmol/L $CoCl_2$ alone group and the RF/6A model cells damaged by 200 μmol/L $CoCl_2$ then irradiated by LED at 7 mW/cm^2, 14 mW/cm^2, and 28 mW/cm^2 groups decreased significantly. But the cell viability of RF/6A model cells damaged by 200 μmol/L $CoCl_2$ and irradiated by LED at 21 mW/cm^2 had no significant difference compared with the control group. Compared with 200 μmol/L $CoCl_2$ alone group, cell viability of RF/6A model cells damaged by 200 μmol/L $CoCl_2$ and irradiated by LED with power intensity of 7, 14, 21, and 28 mW/cm^2 increased significantly. Here, 21 mW/cm^2 was chosen as the optimum power density for the following up experiment (Figure 2).

3.3. Effect of LED Treatment on Cytochrome C Oxidase Activity. As shown in Figure 3, compared with control group, cytochrome C oxidase activity of cells damaged by 200 μmol/L $CoCl_2$ decreased significantly and cytochrome C oxidase activity of RF/6A model cells damaged by 200 μmol/L, $CoCl_2$ for 24 h and irradiated by 21 mW/cm^2 LED decreased significantly. There are no significant differences between the cytochrome C oxidase activity of RF/6A model cells treated by 21 mW/cm^2 LED and control group. However, compared with 200 μmol/L $CoCl_2$ alone group, cytochrome C oxidase activity of RF/6A model cells damaged by 200 μmol/L $CoCl_2$ and irradiated by 21 mW/cm^2 LED increased significantly although it did not completely reverse the cytochrome C oxidase to the level of control group. There is significantly difference between the cytochrome C oxidase activity of RF/6A model cells irradiated by 21 mW/cm^2

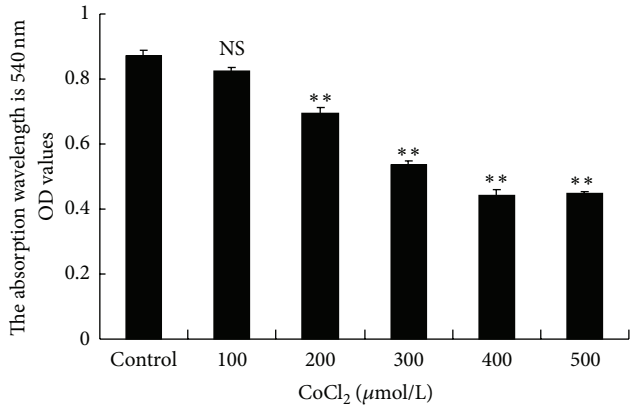

Figure 1: Effect of $CoCl_2$ with various concentrations on cell viability of RF/6A model cell. $^{**}P < 0.01$ indicates comparison with control group.

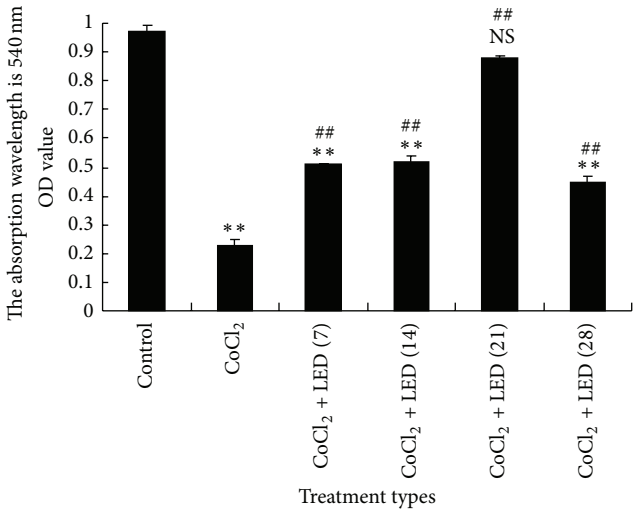

Figure 2: Effects of 670 nm LED irradiation with various power densities on cell viability of RF/6A cells incubated with $CoCl_2$ at 200 μM for 24 h. Data are means ± SEM. $^{**}P < 0.01$ indicates comparison with control group. NS indicates no significance; $^{\#\#}P < 0.01$ indicates comparison with 200 μmol/L $CoCl_2$ group.

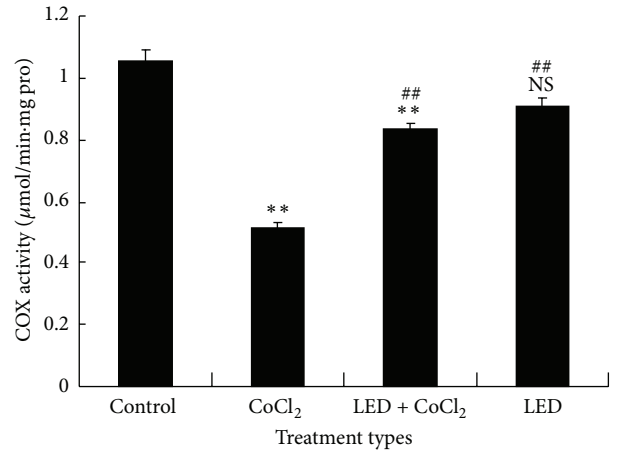

Figure 3: Effect of 21 mW/cm^2 670 nm LED treatment on cytochrome C oxidase activity. Data are means ± SEM. $^{**}P < 0.01$ indicates comparison with control group. NS indicates no significance compared with control group. $^{\#\#}P < 0.01$ indicates comparison with 200 μmol/L $CoCl_2$ group.

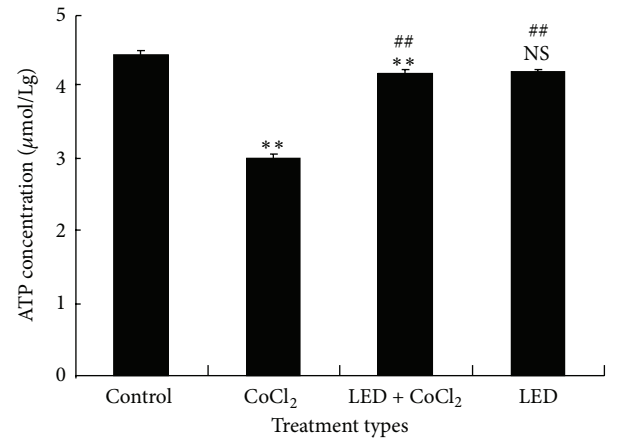

Figure 4: Effects of 21 mW/cm^2 670 nm LED treatment on ATP content. Data are means ± SEM. $^{**}P < 0.01$ indicates comparison to control group. NS indicates no significantly difference compared with control group. $^{\#\#}P < 0.01$ indicates comparison to $CoCl_2$ group.

670 nm LED treatment alone group and 200 μmol/L $CoCl_2$ alone group.

3.4. Effect of LED Treatment on ATP Content. It was seen that, compared with control group, the ATP content of RF/6A model cells damaged by 200 μ mol/L, $CoCl_2$ for 24 h was reduced significantly (Figure 4); the ATP content of RF/6A model cells damaged by 200 μmol/L, $CoCl_2$ for 24 h and irradiated by 21 mW/cm^2 LED decreased significantly. There are no significant differences between the ATP content of RF/6A model cells treated by 21 mW/cm^2 LED and control group. But compared with 200 μmol/L $CoCl_2$ alone group, ATP content of RF/6A model cells damaged by 200 μmol/L $CoCl_2$ and irradiated by LED with power intensity of 21 mW/cm^2 increased significantly. There is significant difference between the ATP content of RF/6A model cells irradiated by 21 mW/cm^2 670 nm LED alone and 200 μmol/L $CoCl_2$ treatment alone.

4. Discussion

Diabetes produces retinal abnormalities that result in damage to the vasculature and neurons, and in severe cases, loss of vision itself. The pathogenesis of DR remains to be elucidated, although reduction in hyperglycemia has been shown to exert positive effects on the development and progression of

diabetic retinopathy. Nevertheless, achievement and maintenance of glycemic control have been difficult or impossible in many patients; therefore effective therapies are explored to inhibit the retinopathy. An alternative approach would be to identify innovative noninvasive treatment modalities that act by multiple potential mechanisms. Light in the spectrum from red to near-infrared region (630–1000 nm) has been reported to be beneficial in the treatment of infected, ischemic, and hypoxic wounds and other soft tissue injuries.

High-energy light has been used as a treatment option for ophthalmic diseases, such as in laser photocoagulation for age-related macular degeneration or diabetic retinopathy. In the present study, however, we demonstrated that photobiomodulation using low-intensity light can recover the damage of hypoxia caused by cobalt chloride. We found that low-intensity 670 nm LED irradiation for 3 days improved cell viability, cytochrome C oxidase activity, and ATP content of hypoxic RF/6A cells damaged by $CoCl_2$ exposure. Our data demonstrated the recovery role of LED irradiation on hypoxia damage of RF/6A cells caused by cobalt chloride. Moreover, it has no effect on normal RF/A6 cells, indicating that there is no side effect of 670 nm LED irradiation, which means low-intensity LED irradiation only plays a recovery role on the cells under pathological state, as Liu et al. have pointed out [11]. Further study for the mechanisms that LED only has effect on the pathological cells but has no effect on normal cells should have been further studied.

Cytochrome C oxidase complex has the antioxidant effect. It is the last enzyme in the respiratory electron transport chain of mitochondria. It receives an electron from each of four cytochrome c molecules and transfers them to one oxygen molecule. As a primary photoreceptor of light in the spectrum from the red to near-IR region, cytochrome C oxidase plays an important role in LED treatment for retina. In the present study, we explored the effects of low-intensity 670 nm LED irradiation on the proliferation, cytochrome C oxidase activity, and ATP concentration for hypoxic RF/6A model cells demonstrating the possible mechanisms underlying photobiomodulation of cell energy metabolism. Even though 670 nm LED irradiation can completely recover the proliferation of hypoxic RF/6A cells to the level of normal RF/6A cells, the cytochrome C oxidase activity and ATP concentration can only partially recover. It suggested that the pathway [12] maintaining proliferation of normal RF/6A cells and the one maintaining LED completely recovered proliferation of the RF/6A cells exposed on $CoCl_2$ were different from each other, but they maintained the same proliferation. Those two pathways are well-known redundant pathways [11].

It was shown that photoreceptors are the most metabolically active cells in the body and the energy required for phototransduction is derived primarily from oxidative metabolism. These signaling events may include the activation of immediate early genes, transcription factors, cytochrome oxidase subunit gene expression, and a host of other enzymes and pathways related to increased oxidative metabolism [13, 14]. Our study shows that low-intensity 670 nm LED treatment could modulate the oxidative metabolism of retina and improve the retinal function via increasing the activity of cytochrome C oxidase which plays a role in inhibiting the development of diabetic retinopathy. Because photobiomodulation has been found to be associated with minimal risk, noninvasive, inexpensive, and easy to administer, it may be a simple adjunct therapy to help inhibit the development of diabetic retinopathy.

5. Conclusions

Our study presented that the hypoxia damage of RF/6A caused by $CoCl_2$ can be completely recovered by low-intensity LED 670 nm irradiation. Photobiomodulation of 670 nm LED may be a new effective method for DR treatment.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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