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Research Article

Effect and Mechanism of 808 nm Light Pretreatment of Hypoxic Primary Neurons

Xin Chen,[1] Qiang-Li Wang,[2] Shuang Li,[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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This study investigated the effect of low intensity 808 nm light pretreatment of hypoxic primary neurons. Cobalt chloride ($CoCl_2$) has been used to induce hypoxic injury in primary mouse cortical neurons. Low intensity 808 nm light was from light-emitting diode (LED). Cells were randomly divided into 4 groups: normal control group, $CoCl_2$-induced group, $CoCl_2$-induced group with 808 nm light irradiation pretreatment, and normal group with 808 nm light irradiation pretreatment. Effect of low intensity 808 nm light on neuronal morphology has been observed by microscope. MTT colorimetric assay has been used to detect the effect of low intensity 808 nm light on neuronal activity. Adenosine triphosphate (ATP) concentration and cytochrome C oxidase (COX) activity has been detected to study the effect of low intensity 808 nm light on neuronal mitochondria function. The results indicated that low intensity 808 nm light pretreatment alone did not affect cell viability, COX activity, and ATP content of neurons and low intensity 808 nm light pretreatment promoted the cell viability, COX activity, and ATP content of neurons with $CoCl_2$ exposure; however, low intensity 808 nm light pretreatment did not completely recover COX activity and cellular ATP content of primary neurons with $CoCl_2$ exposure to the level of the normal neurons.

1. Introduction

Stroke, known medically as a cerebral vascular accident, could cause the rapid loss of brain function due to disturbance in the blood supply to the brain [1]. The high incidence and mortality of stroke have brought serious harm to people's health [2]. Currently, thrombolytic therapy is used commonly, but it has a strict time window constraint; meanwhile it has the risk of secondary hemorrhage [3]. A number of new and efficient methods are being explored. Among them, the transcranial near-infrared laser therapy (TLT) has been demonstrated to be effective and safe [4–6]. TLT is based on the effect of photobiomodulation of far- or near-infrared light. Biological effect of photobiomodulation using low intensity 808 nm LED light [7] irradiation showed no significant difference with low intensity laser irradiation, but with lower cost. Here, we investigated the biological effect of low intensity 808 nm LED light irradiation on neurons and explored its mechanisms. Our study observed the effect of low intensity 808 nm LED light pretreatment on the cell morphology, cell viability, COX activity, and ATP level of primary neurons with $CoCl_2$ exposure. This study demonstrated the protective function of low intensity 808 nm LED light pretreatment on anoxia injury neurons from the cellular level, which may be useful for the development of nondrug therapy modality of the acute ischemic stroke (AIS).

2. Materials and Methods

2.1. Culture of Primary Neurons. Postnatal Sprague-Dawley rats were brought from Shanghai Laboratory Animal Resources Center. After cutting the skull and removing the brain,

cortical neurons derived from newborn rats (24 h) were dissociated in DMEM/F12 with 10% fetal bovine serum and 10% horse serum and then plated on poly-L-Lysine coated 24-well plates (2×10^5/mL). Cultures were kept at 37°C in a humidified CO_2 incubator. Nonneuronal cell division was halted by exposure to 5 μM Cytarabine for one day. Subsequently, partial medium replacement was carried out every two or three days. After cultured for 7 days, neurons were used for the follow-up experiments.

2.2. Design of the Study. Neurons were divided into four groups:

control group: neurons without any treatment;

$CoCl_2$ group: neurons without any treatment for 3 days then exposed to 100 μM/L-$CoCl_2$ for 12 hours;

LED + $CoCl_2$ group: neurons irradiated for 80 seconds in the dark with 25 mW/cm^2 808 nm LED light, once a day for 3 days, then exposed to 100 μM/L-$CoCl_2$ for 12 hours;

LED group: neurons were irradiated by 25 mW/cm^2 808 nm LED light for 3 days then exposed for 12 hours without 100 μM/L-$CoCl_2$ treatment.

2.3. Cellular Morphology. Cellular morphology was observed by inverted microscopy (Olympus ZX71).

2.4. Cell Viability. Cell viability was determined by the typical MTT assay [8]. The brief process is as follows: cells in good conditions were incubated on 96-well plates, 100 μL each well; MTT working solution was filtered through a 0.22 μm filter and added 20 μL each well. After cultivation in incubator at 37°C for 4 h, the culture medium was removed and then 200 μL of DMSO was added after 10 min shaking. The cells were transferred to ELISA Reader and the cell viabilities were measured by optical density values at 570 nm.

2.5. COX Assays. COX was determined according to the instruction of mitochondrial cytochrome C oxidase activity assay kit. After the cells being digested and collected, mitochondria isolation assay was added, containing protein inhibitor. Stand for 15 minutes after mixing, cells were homogenized using ultrasound. After being centrifuged (600 g) for 10 minutes at 4°C, supernatant was collected. After being centrifuged (11000 g) at 4°C for 10 min, the precipitation was collected. Mitochondrial lysate was added into the prepared mitochondria to obtain the samples. After the addition of sample into the reaction system including cytochrome C and then mixed quickly, the activity of COX was tested by the OD values in 0 s and 60 s from spectrophotometer at 550 nm and then calculated by the protein concentration of samples.

2.6. ATP Content Assays. Using a modification of the luminescence method of Strehler [9], ATP content was measured as follows: after lysis of neuron cells being placed on ice and centrifuged (12000 g) at 4°C for 8 min, supernatant was collected. Some samples were placed in the ice box and the corresponding BCA solution was prepared. The dissolved standard proteins were added to the standard hole of 96-hole plate. An appropriate volume of samples was also added to the same hole and then diluted to 20 μL with PBS solution. Working solution prepared previously was added. The volume of each hole is 200 μL. After being put at 37°C for 30 minutes, the protein concentration of each sample was calculated. 100 μL ATP testing reagent was added into the testing wells and then put at room temperature for 3–5 min so as to exhaust the ATP in background. Then, ATP content can be calculated by liquid scintillation counter and standard curve made previously.

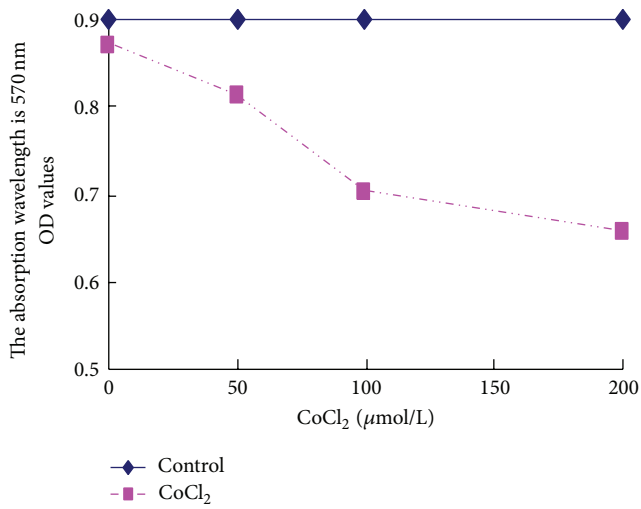

Figure 1: Effects of $CoCl_2$ with various concentrations on cell viability in cultured neurons.

2.7. Statistical Analysis. All light-emitting diode irradiation experiments and biochemical assays associated with measuring changes as a result of light irradiation were performed six times ($n = 6$). 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. Results

3.1. To Determine the Optimal Concentration of $CoCl_2$. Figure 1 shows the cell viability of neurons affected by $CoCl_2$ with different concentration. Compared with normal control group (without any treatment), cell viability began to decrease under exposure to $CoCl_2$ at the concentration of 50 μM/L. Cell viability was 80% of the control group at the concentration of 100 μM/L-$CoCl_2$. Cell viability was less than 50% at the concentration of 200 μM/L-$CoCl_2$. Hence, 100 μM/L-$CoCl_2$ was chosen to produce cell injury in the follow-up experiments.

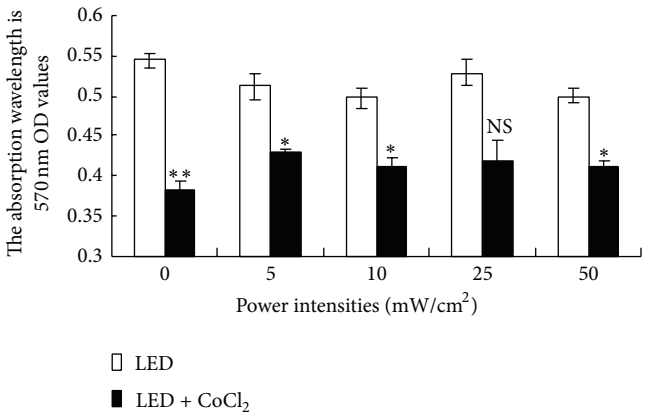

Figure 2: Effect of 808 nm LED light pretreatment with various power densities on cell viability in cultured neurons with 100 μM/L-$CoCl_2$. Neurons were cultured in incubator at 37°C for 4 h, the culture medium was removed, and then 200 μL of DMSO was added after 10 min shaking before cell viability was measured. The power intensities chosen were 5, 10, 25, and 50 mW/cm^2. Data are Means ± SEM. ** means that this group was highly significantly different from control group ($P < 0.01$). * means that this group has statistical difference with control group ($P < 0.05$).

3.2. To Determine the Optimal LED Power Density. The effect of LED light pretreatment with various power densities on cell viability of cultured neurons with $CoCl_2$ is shown in Figure 2. Among the four power densities (5, 10, 25, and 50 mW/cm^2) of LED light pretreatment groups there was nonsignificant difference between cell viability of 25 mW/cm^2 LED light pretreatment group alone and the 25 mW/cm^2 LED light pretreatment with 100 μM/L-$CoCl_2$ group. It means that LED light pretreatment with power intensity of 25 mW/cm^2 has the best capacity of inhibiting the damage induced by $CoCl_2$. The optimal power density for increasing cell viability was 25 mW/cm^2. Thus, the dose of 25 mW/cm^2 was chosen for the following experiment.

3.3. Effect of LED Light Treatment on Neuronal Morphology. The typical morphology of neurons which were irradiated at the optimal power density of 25 mW/cm^2 could be observed (Figure 3). The neuronal soma of control group was full and surrounded by halos; the neuritis were slender and interrelated to a network (Figure 3(a)). The cell body of neuron exposed to 100 μM/L-$CoCl_2$ had serious shrinkage, the neuritis was fractured and the network disappeared, and even the cell died after ruptures (Figure 3(b)). The morphology of the neurons treated with low intensity 808 nm LED light pretreatment with $CoCl_2$ was improved obviously (Figure 3(c)) and the neurons only treated with 808 nm LED light grew in good condition (Figure 3(d)).

3.4. Effect of LED Light Pretreatment on COX Activity. Compared with the control group, the COX activity of $CoCl_2$ group decreased very significantly ($P < 0.01$) and LED + $CoCl_2$ group decreased significantly ($P < 0.05$). There is no significant difference between the COX activity of LED group with control group. Compared with 100 μM/L-$CoCl_2$ group, COX activity of both LED + $CoCl_2$ group and LED group increased significantly ($P < 0.01$) (Figure 4).

3.5. Effects of LED Light Treatment on Cellular ATP Content. Compared with the control group, the cellular ATP content of $CoCl_2$ group decreased very significantly ($P < 0.01$) and LED + $CoCl_2$ group decreased significantly ($P < 0.05$). There is no significant difference between the cellular ATP of LED group with control group. Compared with 100 μM/L-$CoCl_2$ group, COX activity of both LED + $CoCl_2$ group and LED group increased significantly ($P < 0.01$) (Figure 5).

4. Discussion

$CoCl_2$ has been demonstrated to induce the hypoxia damages of cells *in vitro* [10]. It demonstrated that the $CoCl_2$-induced hypoxic injury neurons model could be used for AIS research. Our results showed that low intensity 808 nm light pretreatment promotes the cell viability, COX activity, and ATP content of neurons with $CoCl_2$ exposure. It means that low intensity 808 nm light pretreatment has the capacity to protect against the hypoxia damage of neurons. It might be due to indirect photobiomodulation of low intensity 808 nm light pretreatment according to Liu et al. [11]. However, there was no significant difference about the cell viability, COX activity, and ATP content between the LED group and control group. This suggested that more parameters need to be further assessed.

As a primary biological photoreceptor in the red to near-IR spectrum, COX plays an important role in photobiomodulation [12]. Our research showed that the cellular ATP content of primary neurons rises or falls in concert with the activity of COX. However, low intensity 808 nm LED light pretreatment could not completely recover COX activity and cellular ATP content of primary neurons exposed to $CoCl_2$ to the level of the normal neurons. It suggested that the pathway maintaining proliferation of normal neurons and the one maintaining proliferation of neurons by LED light T pretreatment then exposed to $CoCl_2$ were different from each other, but they maintained the same proliferation [13]. Those two pathways are well-known redundant pathways [11, 14]. Of course, it should be further studied.

5. Conclusion

Under the conditions in which this study was carried out, it was possible to conclude that low intensity 808 nm LED light pretreatment has the capacity to promote mitochondrial energy metabolism and protect against the hypoxia damage of neurons. Low intensity 808 nm LED light pretreatment can improve and restore the neuron function of ischemic penumbra in patients with AIS. Our findings provided experimental evidences for clinical application of low intensity 808 nm LED device.

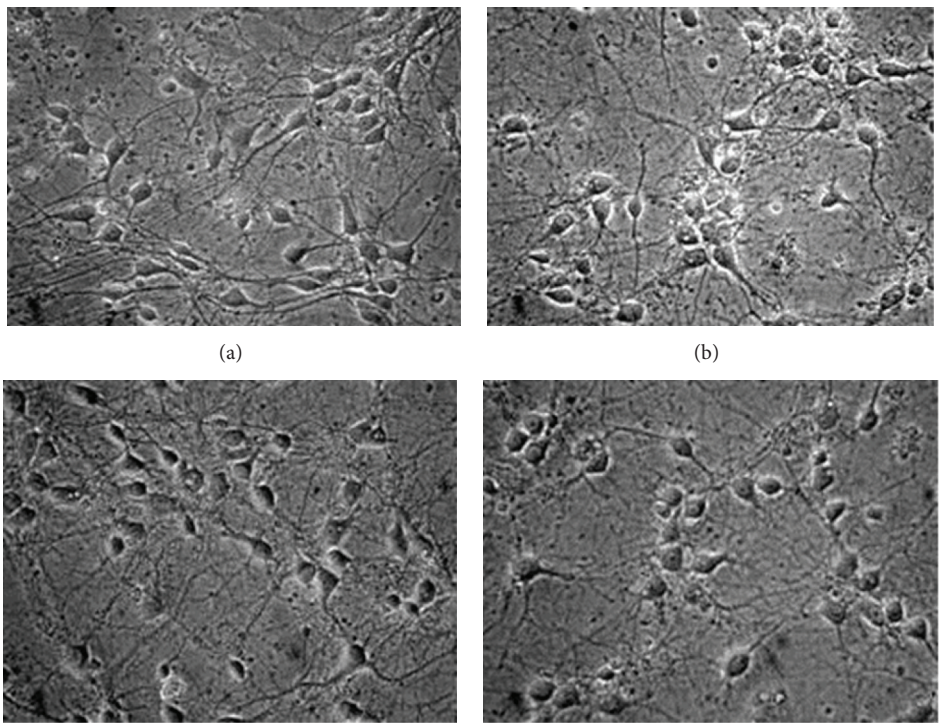

Figure 3: Morphology of the primary cortical neurons in culture (400X). (a) Control, (b) Neurons without any treatment for 3 days then exposed to 100 μM/L-$CoCl_2$ for 12 hours. (c) Neurons irradiated for 80 seconds in the dark with 25 mW/cm^2 808 nm LED light, once a day for 3 days, then exposed to 100 μM/L-$CoCl_2$ for 12 hours. (d) Neurons were irradiated by 25 mW/cm^2 808 nm LED light for 3 days then exposed for 12 hours without 100 μM/L-$CoCl_2$ treatment. Images shown are representative of several plates for each group.

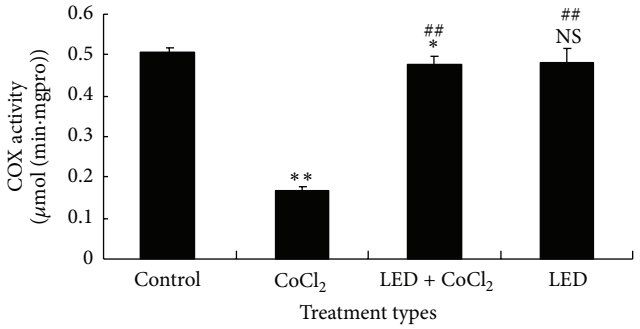

Figure 4: Effect of 25 mW/cm^2 808 nm LED light pretreatment on COX activity. After cultured neuron cells being digested and collected, mitochondria isolation assay was added. 15 minutes later, cells were homogenized. After being centrifuged (600 g) for 10 minutes, supernatant was collected. After being centrifuged (11000 g) for 10 minutes, the precipitation was collected. Mitochondrial lysate was added to obtain the samples. Then COX was measured. Data are Means ± SEM. $^{**}(P < 0.01)$ and $^{*}(P < 0.05)$ mean compared with control group; $^{\#\#}(P < 0.01)$ and $^{\#}(P < 0.05)$ mean compared with 100 μM/L $CoCl_2$ group.

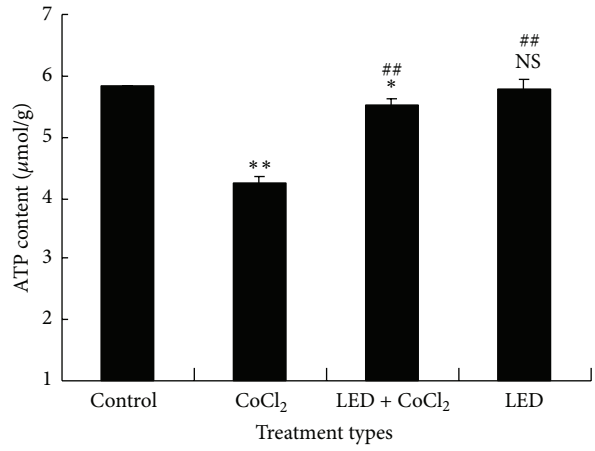

Figure 5: Effect of 25 mW/cm^2 808 nm LED light pretreatment on cellular ATP content. After the lytic neuron cells were placed on ice and centrifuged for 8 min, its supernatant was collected. After treatment and putting at 37°C for 30 minutes, protein concentration of each sample was calculated. After 100 μL ATP testing reagent was added into the testing wells and put at room temperature for 3–5 min, ATP content was calculated. Data are Means ± SEM. $^{**}(P < 0.01)$ and $^{*}(P < 0.05)$ mean compared with control group and $^{\#\#}(P < 0.01)$ and $^{\#}(P < 0.05)$ mean compared with 100 μM/L-$CoCl_2$ group.

Conflict of Interests

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

Acknowledgments

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