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Effect of linear polarized light radiation on impaired mitochondrial oxidative phosphorylation in skeletal muscle

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Abstract

Purpose. The aim of this study was to investigate the effect of linear polarized light radiation (LPLR) on mitochondrial oxidative phosphorylation impaired by hemorrhagic shock or *Escherichia coli* lipopolysaccharide (LPS) in skeletal muscle.

Methods. We studied the effect of LPLR on mitochondrial function of skeletal muscle by using a model of mitochondria impaired by hemorrhage or LPS. The oxygen uptake in states 3 and 4, the respiratory control ratio (RCR), and the adenosine diphosphate-to-oxygen ratio (ADP/O) were measured with a Clark oxygen electrode.

Results. Oxygen uptake in states 3 and 4, RCR, and ADP/O were significantly decreased by hemorrhage for 4 h and by LPS treatment for 12 h. Oxygen uptake in states 3 and 4 impaired by hemorrhage increased significantly from 40.1 ± 3.2 to 60.1 ± 5.4 nmol $O_2 \cdot min^{-1} \cdot mg\ protein^{-1}$ after LPLR, and oxygen uptake in state 4 decreased significantly from 22.8 ± 2.4 to 17.7 ± 1.5 nmol $O_2 \cdot min^{-1} \cdot mg\ protein^{-1}$ after LPLR. RCR and ADP/O were also significantly increased from 1.8 ± 0.3 and 0.9 ± 0.2 to 3.4 ± 0.3 and 1.5 ± 0.1, respectively, by LPLR. Oxygen uptake in states 3 and 4 impaired by LPS was improved from 46.6 ± 5.1 and 21.0 ± 1.9 to 53.8 ± 6.2 and 17.9 ± 2.3 nmol $O_2 \cdot min^{-1} \cdot mg\ protein^{-1}$, respectively following LPLR. RCR and ADP/O were also elevated significantly from 2.2 ± 0.2 and 0.9 ± 0.2 to 3.0 ± 0.3 and 1.4 ± 0.2, respectively, after LPLR.

Conclusion. LPLR improved mitochondrial oxidative phosphorylation of skeletal muscle impaired by hemorrhagic shock or *E. coli* LPS.

Key words Linear polarized light therapy · Mitochondrial function · Skeletal muscle · *Escherichia coli* lipopolysaccharide and hemorrhage

Introduction

Linear polarized light radiation (LPLR) can provide infrared rays with wavelengths ranging from 600 to 1600 nm. The waves are easily absorbed in the body to 50 mm in depth with an output of 1800 mW [1]. The equipment seems to activate the organism by electromagnetism as well as low-level reactive laser. LPLR therapy has been widely employed because it has been shown to be effective in the treatment of pain, including complex regional pain syndrome (CRPS) [2] and musculoskeletal pain [3]. It has been demonstrated that chronic pain, including CRPS, is associated with impairment of oxygen extraction and high-energy phosphate metabolism [4] in skeletal muscles. These dysfunctions of energy metabolism appear to be caused by mitochondrial dysfunction, because mitochondria play an important role in energy metabolism of skeletal muscle and the dysfunction causes cellular damage. Decreased production of adenosine triphosphate (ATP) because of mitochondrial dysfunction increases muscle irritability and leads to skeletal muscle injury [5].

LPLR is reported to increase blood flow and temperature in human skin [6], but the mechanism remains unclear. The high output power, action of light, and photochemical effect under LPLR would have a biologically stimulatory action, including stimulation of mitochondria, probably leading to reenergization of cells. The present study was designed to investigate the effect of LPLR on the mitochondrial function of guinea pig skeletal muscle injured by hemorrhage or intraperitoneal injection of *Escherichia coli* lipopolysaccharide (LPS).

Materials and methods

Forty male guinea pigs weighing 300 to 400 g were the subjects of the study. On the day prior to the study, the

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animals were anesthetized with 40 mg·kg^{-1} of intraperitoneal pentobarbital, and catheters were inserted into a carotid artery and a femoral artery for measurement of blood pressure. The animals were divided into five groups of eight. Group 1 was treated with saline only as control. Group 2 was subjected to hemorrhagic shock for 4 h. Group 3 was irradiated with LPLR after 4 h of hemorrhagic shock. Group 4 was treated with LPS for 12 h. Group 5 was irradiated with LPLR after LPS treatment for 12 h. Hemorrhagic shock was induced by Wigger's procedure [7]. Blood was drawn into a reservoir through a cannula in a femoral artery until the mean blood pressure was 30 mmHg. The pressure was kept constant for 4 h by controlling the amount of blood in the reservoir. *E. coli* lipopolysaccharide B_8 (Difco, St. Louis, MO, USA), 10 mg·kg^{-1}, was administrated intraperitoneally as LPS treatment. The dose of *E. coli* was confirmed to produce 50% lethality after 24 h in the preliminary study.

Mitochondria of skeletal muscle of the guinea pig were isolated according to the modified method of Chappell et al. [8]. Under anesthesia with pentobarbital, skeletal muscles of the right hind limb were removed to isolate mitochondria and were put in an ice-cold medium consisting of 0.25 M sucrose and 0.1 mM ethylenediamine tetra-acetate (EDTA), pH 7.4. After removal of cell debris and blood, slices approximately 0.2 mm thick were minced with scissors. After the slices had been washed three times with the same medium, the suspension was homogenized in a homogenizer with a very loosely fitting pestle for 30 s. The homogenate was centrifuged at 400× *g* for 10 min, and the supernatant solution was carefully decanted. The supernatant solution was then centrifuged at 12000× *g* for 10 min. The sediment was resuspended in 20 ml of the same medium and centrifuged at 6500× *g* for 10 minutes. The loosely packed white pellet was removed completely by shaking the tube with a small volume of medium. The white pellet contains mostly mitochondria without microsomes, nuclei, and other components, and the mitochondria have biologically high activity [9].

Isolated mitochondria were directly irradiated with LPLR to investigate the direct effect of LPLR on the mitochondrial function of skeletal muscle. A model HA-550 (KSK Tokyo Iken, Tokyo, Japan) was used for LPLR. The radiation was applied to isolated mitochondria directly after LPS exposure for 12 h and hemorrhagic shock for 4 h, with a cycle of 1 s on and 2 s off at an output of 1800 mW for 10 min at a distance of 5 cm.

Mitochondrial respiration activity was assayed by using a Clark oxygen electrode (OBH-100, Ohtsuka Denshi, Osaka, Japan) to measure oxygen utilization by the mitochondria in the presence of succinate as a substrate. This equipment can measure dissolved oxygen as electric current with a platinum electrode by the polarographic method. The incubation medium consisted of 0.3 M mannitol, 0.01 M KCl, 0.01 M Tris-HCl buffer, 0.005 M phosphate buffer, 0.2 mM EDTA, and 0.25 mM ADP in a total volume of 1 ml (pH 7.4). After 5 mM glutamate was added to the medium as substrate, oxygen uptake in states 3 and 4 was measured. The respiration that occurs with substrate and ADP in the medium is state 3, in which mitochondria shows active respiration. The respiration that occurs with substrate but with no ADP in the medium is state 4, in which mitochondria shows stable respiration. The respiratory control ratio (RCR) and adenosine diphosphate to oxygen ratio (ADP/O) were calculated according to the method of Chance and Williams [10]. RCR is the ratio of state 3 respiratory rate to state 4 rate. ADP/O is the ratio of the added ADP to oxygen uptake in state 3.

Data were expressed as means ± SD. Mitochondrial respiration functions among the groups were analyzed by analysis of variance with the Bonferroni correction. Differences in mean values were considered significant when $P < 0.05$.

Results

The temperature of the medium including mitochondria did not significantly increase after LPLR (22.7 ± 0.06°C before and 22.9 ± 0.05 after LPLR).

Figures 1 and 2 show the effects of LPLR on the mitochondrial function damaged by hemorrhage and LPS. Oxygen uptake in state 3 significantly ($P < 0.001$) decreased, and oxygen uptake in state 4 significantly ($P < 0.001$) increased 4 h after hemorrhage and 12 h after LPS. RCR and ADP/O were significantly ($P < 0.001$) decreased by hemorrhage and LPS.

Oxygen uptake in state 3 decreased by hemorrhage was significantly ($P < 0.001$) increased by LPLR from 40.1 ± 3.2 to 60.1 ± 5.4 nmol O_2·min^{-1}·mg protein^{-1}, and oxygen uptake in state 4 increased by hemorrhage was significantly ($P < 0.01$) decreased from 22.8 ± 2.4 to 17.7 ± 1.5 nmol O_2·min^{-1}·mg protein^{-1} after LPLR. RCR and ADP/O diminished by hemorrhage were significantly ($P < 0.01$) increased from 1.8 ± 0.3 and 0.9 ± 0.2 to 3.4 ± 0.3 and 1.5 ± 0.1, respectively, by LPLR.

Oxygen uptake in states 3 and 4 impaired by LPS was improved from 46.6 ± 5.1 and 21.0 ± 1.9 to 53.8 ± 6.2 and 17.9 ± 2.3 nmol O_2·min^{-1}·mg protein^{-1}, respectively, following LPLR. RCR and ADP/O were also significantly ($P < 0.01$) elevated from 2.2 ± 0.2 and 0.9 ± 0.2 to 3.0 ± 0.3 and 1.4 ± 0.2, respectively after LPLR. RCR and ADP/O in LPS after LPLR were significantly different from those of controls, but not in hemorrhage.

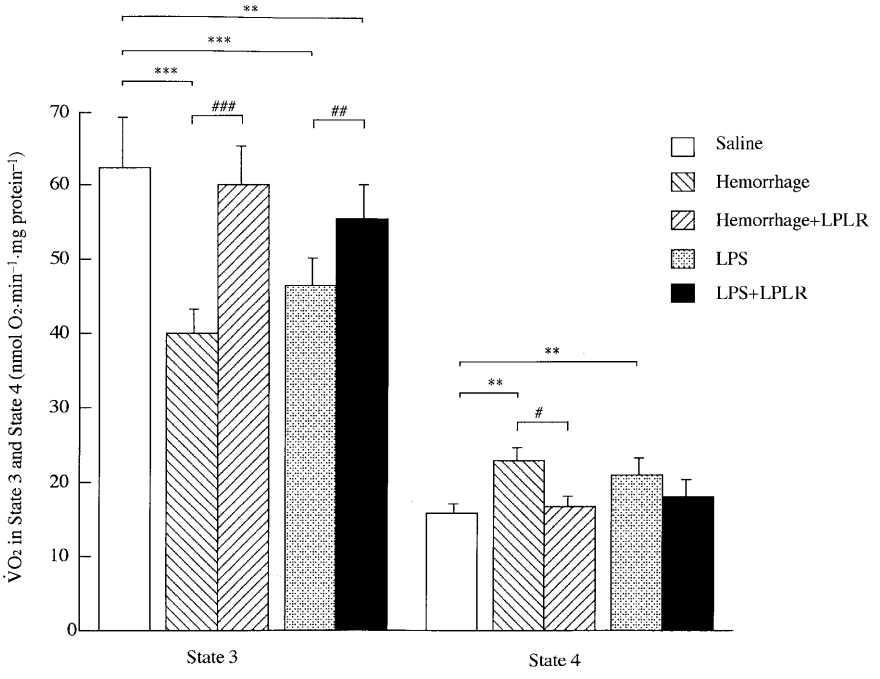

Fig. 1. Effect of linear polarized light radiation (*LPLR*) on oxygen uptake in states 3 and 4 of skeletal muscle mitochondria following hemorrhage and *Escherichia coli* lipopolysaccharide (*LPS*) in guinea pigs. Data are expressed as means ± SD. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ with *vs* without LPLR. ** $P < 0.01$, *** $P < 0.001$ *vs* saline (control)

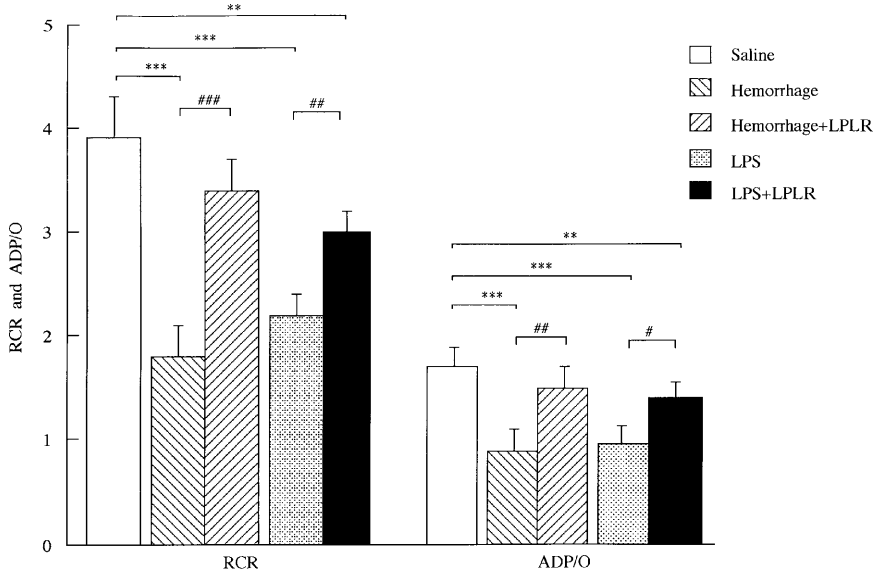

Fig. 2. Effect of linear polarized light (*LPLR*) on oxygen uptake in respiratory control ratio (*RCR*) and adenosine diphosphate-to-oxygen ratio (*ADP/O*) of skeletal muscle mitochondria following hemorrhage and *Escherichia coli* lipopolysaccharide (*LPS*) in guinea pigs. Data are expressed as means ± SD. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ with *vs* without LPLR. ** $P < 0.01$, *** $P < 0.001$ *vs* saline (control)

Discussion

In this study, LPLR improved mitochondrial respiration of oxygen uptake in states 3 and 4, RCR, and ADP/O impaired by hemorrhagic shock and LPS. The results demonstrate that LPLR is useful for improving damaged mitochondria of skeletal muscle.

The alterations in mitochondrial energy-linked functions are demonstrated in both hemorrhagic and endotoxin shock [11]. The decrease of oxygen uptake in state 3 is related to the decline in ADP utilization rate together with the lack of ADP-stimulated cytochrome response and respiration. Increased oxygen uptake in state 4 indicates uncoupling response of mitochondrial respiration [12]. RCR reflects the state of mitochondrial integration, and ADP/O indicates mitochondrial oxidative phosphorylation. The changes in these parameters in hemorrhagic and endotoxin shock are chiefly caused by tissue hypoxia according to hypoperfusion [11]. However, we found an effect of LPLR on mitochondria

per se. Our results indicate that LPLR would directly influence mitochondria to improve oxidative phosphorylation independently of the circulation.

The mechanism of improvement in oxidative phosphorylation by LPLR remains unclear. LPLR is supposed to have the action of light, radiation heat activity, and photochemical activity and activates the organism by electromagnetism. We could not find a significant change in the temperature of the solution including mitochondria after LPLR. Thus, the beneficial effect of LPLR on mitochondria will be associated with photochemical activity with LPLR. In this study, we found a direct action on mitochondria of skeletal muscles by LPLR. Skeletal muscles are energy engines. They convert the energy stored ATP to mechanical movement. If anything interferes with the production of ATP in skeletal muscle, the muscle increases irritability to compromise the energy function, leading to skeletal muscle pain [5]. The beneficial effect of LPLR on mitochondrial oxidative phosphorylation of the skeletal muscle may relieve skeletal muscle pain.

In this study, LPLR was applied with a cycle of 1 s on and 2 s off at an output of 1800 mW for 10 min and a distance of 5 cm. Because LPLR was applied clinically with a cycle of 1 s on and 2 s off, we used the same cycle. It was difficult to decide what power output should be used for isolated mitochondria. The degree of power output that reaches skeletal muscle is unknown. Probably, the power output will be lower than 1800 mW. On the other hand, because the wave of 0.6–1.6 μm with LPLR is not much absorbed by water, the power output applied to isolated mitochondria will be less than 1800 mW. Thus, we decided to irradiate at an output of 1800 mW. We irradiated isolated mitochondria directly to investigate the direct effect of LPLR on mitochondria of skeletal muscle. It may be clinically appropriate to isolate the mitochondria after LPLR in vivo, but the method will have an influence on blood flow. Therefore, we directly irradiated the isolated mitochondria.

The effects of LPLR on RCR and ADP/O differed between hemorrhage and LPS. LPLR was more effective in hemorrhagic shock than in LPS. In skeletal muscle injected with endotoxin, protein accumulation is seen in the interstitial edema, but not in hemorrhagic shock [12]. The cellular damage by endotoxin includes a direct toxic effect of the injected endotoxin or the effects of mediators such as elastase, cytokine, arachidonic acid, or oxygen radicals [13]. The present study indicates that LPLR may have less influence on mitochondria injured by chemical mediators or oxygen radicals. Chronic pain causes a decrease of the blood flow in its early stage and later release of several chemical mediators. Cells treated by LPS are likely to be damaged not only by decrease in blood flow, but also by chemical mediators. This indicates that LPLR may be more effective in the early stage of chronic pain than in the delayed stage. In this study, we did not study the effect of LPLR on normal mitochondria. Normal mitochondrial function is affected by concentrations of ATP, ADP, substrate, and intracellular Ca^{2+}. Thus, it is possible that LPLR also activates the function of normal mitochondria, if LPLR changes intracellular metabolism.

In summary, radiation of LPLR produced beneficial effects on mitochondrial oxidative phosphorylation of skeletal muscle damaged by hemorrhage or *E. coli* LPS. The effect was more pronounced in hemorrhage than in *E. coli* LPS.

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