

LED ENHANCEMENT IN MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION FOR HEPATECTOMIZED RATS¹

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ABSTRACT

Recently, the LED (light emitting diode) developed by the Optics Group of IFSC-USP has been used instead of laser for the treatment of skin tumors by the PDT (Photodynamic Therapy) because of its low operational cost compared to the use of a laser. In this paper we investigate the effect of LED light on oxidative phosphorylation during liver regeneration after partial hepatectomy. Twenty-four male Wistar rats (250 g) were kept in identical housing units on a 12-hour light/12 hour dark cycle. The LED 10 group was exposed to LED at 638 nm (10 J/cm² for 3 minutes). Seventy percent partial hepatectomy was performed in the LED 10 and HPC (Partial Hepatectomy-Control). A sham-operated group (C) was used for control. Twenty four hours after the procedure, LED 10, HPC and control animals were sacrificed. Samples of liver tissue were used for the mitochondrial respiration assay. Statistical comparisons of the groups were performed by analysis of variance (ANOVA), followed by the Bonferroni post-test. Probability values less than 0.05 were considered to be statistically significant. the phosphorylation index (FI) for the LED 10 group was higher than that for the HPC group and for the sham group (p<005). The FI for the HPC group was higher than that for the sham group (p<005). The values of the ADP:O ratio for the three groups, which did not differ significantly from one another (p > 0.05). In the

present study we noted an effective interaction between LED light and hepatic mitochondria, with an increased phosphorylation rate for the latter. Available from: URL: <http://www.scielo.br/acb>

Key Words - Liver; mitochondrial function; phosphorylation index; partial hepatectomy.

INTRODUCTION

Many studies have demonstrated that laser light modifies cell metabolism.^{1,2,3} Both visible red light and infrared light have been shown to present many different effects at the cellular level. Light radiation must be absorbed⁴ to promote a biological response; on this basis, we expected any type of light therapy to be an effect of light itself, and not a function of coherency which is a unique property of laser light.^{2,5} Thus, almost monochromatic light is expected to present effects similar to those of laser. With this in mind, we performed a series of experiments to demonstrate that the effects of low energy laser therapy are due to the effects of light and not to the unique properties of lasers.^{2,3,5} The experiment here reported is a first and involves the observation of variations of respiratory cell function after irradiation with a light emitting diode (LED). Recently, the LED developed by the Optics Group of IFSC-USP has been used instead of laser for the treatment of skin tumors by the PDT (Photodynamic

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Therapy) (an effect not previously tested by other investigators) because of its low operational cost compared to the use of a laser. In this paper we investigate the effect of LED light on oxidative phosphorylation during liver regeneration after partial hepatectomy.

METHODS

Animals

Twenty-four male Wistar rats weighing 250 g were obtained from the Central Animal House of the University of São Paulo, Ribeirão Preto, Brazil. The animals received standard laboratory chow (Purina) and water *ad libitum*, and were kept in identical housing units on a 12-hour light/12 hour dark cycle. The LED 10 group was exposed to LED at 638 nm (10 J/cm² for 3 minutes). Seventy percent partial hepatectomy was performed by the method of Higgins & Anderson⁶ in the LED 10 and HPC (Partial Hepatectomy-Control). A sham-operated group (C) was used for control.

On the basis of a previous study by our group,^{5,7} 24 hours after the procedure, LED 10, HPC and control animals were sacrificed under diethyl ether anesthesia. Samples of liver tissue were used for the respiration assay.

Isolation of Liver Mitochondria⁸

Mitochondria were isolated by conventional differential centrifugation. Fragments of liver tissue were washed in cold saline and homogenized three times at 1 min intervals in a Potter-Elvehjem homogenizer in 10 mL of a medium containing 250 mM sucrose, 1 mM EGTA, and 10 mM HEPES-KOH, pH 7.2. Homogenates were centrifuged at 770 g for 5 min and the resulting supernatant was further centrifuged at 9800 g for 10 min. Pellets were suspended in 10 mL of a medium containing 250 mM sucrose, 0.3 mM EGTA and 10 mM HEPES-KOH, pH 7.2, and centrifuged at 4500 g for 15 min. The final mitochondrial pellet was suspended in 0.5 ml of a medium containing 250 mM sucrose and 10 mM HEPES-KOH, pH 7.2. All procedures were conducted at 4°C and all solutions were prepared using glass-distilled and deionized water.

Mitochondrial Respiration⁹

Mitochondrial respiration was monitored polarographically with an oxygraph equipped with a Clark-type oxygen electrode (IFSC-USP). Assays were performed at 30°C using mitochondria energized with

5 mM potassium succinate. Respiration media contained 125 mM sucrose, 65 mM KCl, 0.1 mM EGTA, 1 mM MgCl₂, 2 mM KH₂PO₄, and 10 mM HEPES-KOH, pH 7.4. The state III of mitochondrial respiration was determined after the addition of 400 nmol of ADP and state IV (basal) was determined after phosphorylation of ADP addition. Both states are expressed as n atoms of O₂/min/mg of protein. The ratio between the state III and IV rates (ratio of respiration control, RCR) was determined and represents the coupling of the mitochondria to the medium. Mitochondrial protein content was determined by the biuret reaction.

Statistical analysis

Data are reported as mean ± SEM. Statistical comparisons of the groups were performed by analysis of variance (ANOVA) for parametric measurements, followed by the Bonferroni post-test. Probability values less than 0.05 were considered to be statistically significant.

RESULTS

As shown in figure 1, the phosphorylation index (FI) (mean + SD) for the LED 10 group was higher than that for the HPC group and for the sham group (p < 0.05). The FI for the HPC group was higher than that for the sham group (p < 0.05).

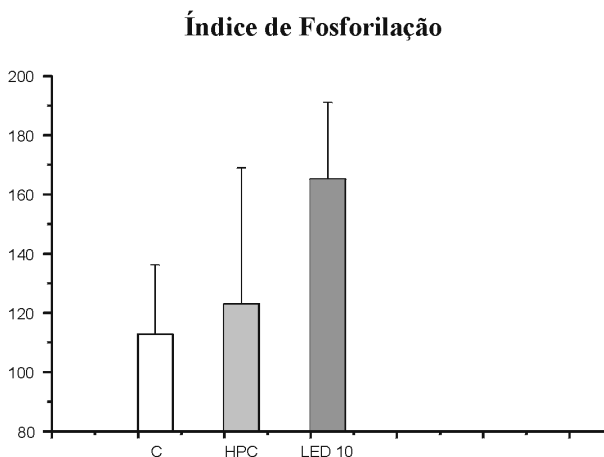


Figure 1 - Phosphorylation Index in the groups Control (C), Partial Hepatectomy-control (HPC) and HPC and Led.

Figure 2 shows the values of the ADP:O ratio (mean + SD) for the three groups, which did not differ significantly from one another (p > 0.05).

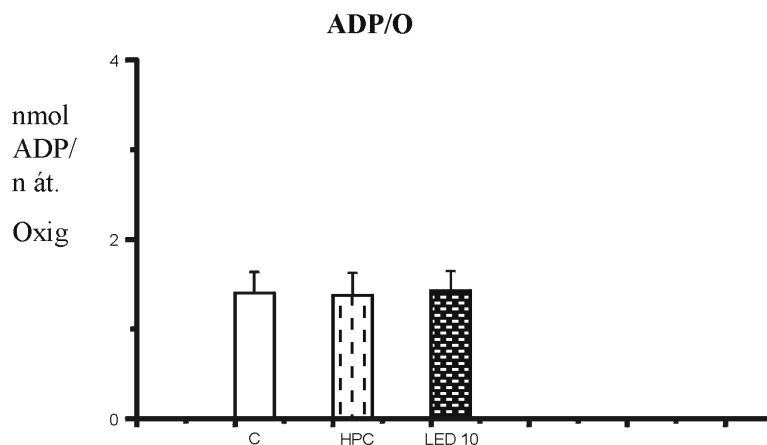


Figure 2 - ADP/O index in the groups Control (C), Partial Hepatectomy-control (HPC) and HPC and Led

Figure 3 shows the LED-based device emitting red light at an emission peak at 626 nm.



Figure 3 - LED-based device emitting red light at an emission peak at 626 nm.

DISCUSSION

Previous studies from our laboratory have shown an increase in the energy capacity and hepatic regeneration of hepatic remnants illuminated with low intensity laser light at all wavelengths used.^{5,10} Laser light is a coherent light with a specific wavelength that effectively stimulates mitochondrial function.^{2,5,10} In the present study we investigated the effect of low intensity LED light on mitochondrial function through the oxidative phosphorylation index. LEDs and lasers both produce radiation at specific wavelength. Nevertheless, LEDs are neither coherent nor collimated and they are broader in emission when compared with lasers. These properties can cause higher penetration in many cases.

We have used a special home-made LED device that employs an array of emitting centers, with wavelength centered at 630 nm. The overall emitted powers over a full hemisphere is about 500 mW, given an energy density that depends on the distance between device and target. Intensities as 20 to 50 mW/cm² are obtained with this device.

In the present study we noted an effective interaction between LED light and hepatic mitochondria, with an increased phosphorylation rate for the latter. This increase occurred at levels similar to those induced by laser light, indicating a possible effect of hepatic regeneration induction, an energy-dependent process.^{2,5,10}

The ADP:O ratio was similar in the three groups studied, showing that LED light did not have a damaging effect on the mitochondrial membrane.¹¹ Thus, the light induced an increase in oxidative phosphorylation without damaging the mitochondrial membrane. Further studies are currently underway in our laboratory for a better understanding of the interaction between LED light and hepatic cells and organelles for clinical application to hepatology in the near future.

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