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Short communication

Evaluation of mitochondrial respiratory chain activity in wound healing by low-level laser therapy

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Abstract

Laser therapy is used in many biomedical sciences to promote tissue regeneration. Many studies involving low-level laser therapy have shown that the healing process is enhanced by such therapy. In this work, we evaluated mitochondrial respiratory chain complexes II and IV and succinate dehydrogenase activities in wounds after irradiation with low-level laser. The animals were divided into two groups: group 1, the animals had no local nor systemic treatment and were considered as control wounds; group 2, the wounds were treated immediately after they were made and every day after with a low-level laser (AsGa, wavelength of 904 nm) for 10 days. The results showed that low-level laser therapy improved wound healing. Besides, our results showed that low-level laser therapy significantly increased the activities of complexes II and IV but did not affect succinate dehydrogenase activity. These findings are in accordance to other works, where cytochrome *c* oxidase (complex IV) seems to be activated by low-level laser therapy. Besides, we showed, for the first time, that complex II activity was also activated. More studies are being carried out in order to evaluate other mitochondrial enzymes activities after different doses and irradiation time of low-level laser.
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Keywords: Low-level laser therapy; Respiratory chain; Wound healing; Metabolism

1. Introduction

Tissue repair is a dynamic interactive process, which involves several biochemical and cellular changes. Laser therapy is used in many biomedical sciences to promote tissue regeneration. Many studies involving the low-level laser therapy have shown that the healing process is enhanced by such therapy [1–4]. In the last years, many researchers have described various important biological effects associated with low-level laser therapy.

It has been shown that low-level laser therapy presents advantages such as the control of pain, anti-inflammatory action, increase of collagen production, fibroblastic proliferation and increase of local microvascularization [5–8]. It has been also demonstrated that such therapy increased cellular metabolism, enhancing the regenerative potential and promoting the anti-inflammatory effect with analgesia and vasodilatation [5,9]. Other studies, however, emphasize that depending on the applied dose, wavelength, irradiation time and also the conditions of the treated tissue, different biological answers can be achieved [10–13].

The aim of this work was to evaluate mitochondrial respiratory chain complexes II and IV and succinate dehydrogenase activities in wounds after irradiation with low-level laser.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (250–300 g) were obtained from Central Animal House of Universidade do Extremo Sul Catarinense. They were caged in group of five with free

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access to food and water and were maintained on a 12-h light-dark cycle (lights on 7:00 am), at a temperature of 23 °C ± 1 °C.

2.2. Ulceration and low-level laser treatment

After anesthesia with ketamine via i.p. (80 mg/kg), the dorsal region of the animals were trichotomyzed following disinfection with alcohol (70%). On the medial dorsal portion a circular area of skin (approximately 8 mm of diameter) was removed with a punch. The animals were divided into two groups: group 1, the animals had no local nor systemic treatment and were considered as control wounds; group 2, the wounds were treated immediately after they were made and every day after with a low-level laser for 10 days. The low-level laser used in this study was a arsenium–gallium (AsGa) with a wavelength of 904 nm, with power ranging from 15 to 30 mW, with total dose per session of 3 J/cm^2. The laser irradiation was performed in five distinct regions around the wound margin [7]. After 10 days of the surgery act and treatment, all animals were sacrificied by decapitation. The obtained pieces were used for mitochondrial enzymes activities evaluation. *In vivo* studies were performed in accordance with National Institutes of Health guidelines and with the approval of Ethics Committee from Universidade do Extremo Sul Catarinense. The efficacy of low-level laser therapy for wound healing was evaluated by measuring the wound size 3 and 10 days after ulceration.

2.3. Tissue and homogenate preparation

Tissue around wound was homogenized (1:10, w/v) in SETH buffer, pH 7.4 (250 mM sucrose, 2 mM EDTA, 10 mM Trizma base, 50 IU/ml heparin). The homogenates were centrifuged at 800 × g for 10 min and the supernatants kept at −70 °C until used for enzyme activity determination. The maximal period between homogenate preparation and enzyme analysis was always less than 5 days. Protein content was determined by the method described by Lowry and colleagues [14] using bovine serum albumin as standard.

2.4. Mitochondrial enzymes activities

The activities of the respiratory chain enzyme complex succinate: DCIP oxireductase (complex II) and succinate: phenazine oxireductase (soluble succinate dehydrogenase (SDH)) were determined according to the methods of Fischer and colleagues [15]. Complex II (succinate: DCIP oxireductase) activity was measured by following the decrease in absorbance due to the reduction of 2,6-DCIP) at 600 nm with 700 nm as reference wavelength ($\varepsilon = 19.1\ \mathrm{mM^{-1}\ cm^{-1}}$). The reaction mixture consisting of 40 mM potassium phosphate, pH 7.4, 16 mM succinate and 8 μM DCIP was preincubated with 40–80 μg homogenate protein at 30 °C for 20 min. Subsequently, 4 mM sodium azide and 7 μM rotenone were added and the reaction was initiated by addition of 40 μM DCIP and was monitored for 5 min. The activity of succinate: phenazine oxireductase (soluble SDH) was measured following the decrease in absorbance due to the reduction of 2,6-DCIP at 600 nm with 700 nm as reference wavelength ($\varepsilon = 19.1\ \mathrm{mM^{-1}\ cm^{-1}}$) in the presence of phenazine methasulphate (PMS). The reaction mixture consisting of 40 mM potassium phosphate, pH 7.4, 16 mM succinate and 8 μM DCIP was preincubated with 40–80 μg homogenate protein at 30 °C for 20 min. Subsequently, 4 mM sodium azide, 7 μM rotenone and 40 μM DCIP were added and the reaction was initiated by addition of 1 mM PMS and was monitored for 5 min. The activity of cytochrome *c* oxidase (complex IV) was measured by the method of Rustin and colleagues [16]. Complex IV activity was measured by following the decrease in absorbance due to the oxidation of previously reduced cytochrome *c* at 550 nm with 580 nm as reference wavelength ($\varepsilon = 19.1\ \mathrm{mM^{-1}\ cm^{-1}}$). The reaction buffer contained 10 mM potassium phosphate, pH 7.0, 0.6 mM *n*-dodecyl-d-maltoside, 2–4 μg homogenate protein and the reaction was initiated with addition of 0.7 μg reduced cytochrome *c*. The activity of complex IV was measured at 25 °C for 10 min.

2.5. Statistical analysis

Data were analyzed by Student's *t*-test. All analyses were performed using the Statistical Package for the Social Science (SPSS) software.

3. Results

In this work, we measured mitochondrial respiratory chain complexes II and IV and succinate dehydrogenase activities in wounds after treatment with low-level laser for 10 days. Fig. 1 shows that low-level laser therapy improves wound healing, by evaluating the wound size 3 ($F(10) = 5.80$, $p < 0.01$) and 10 days ($F(10) = 5.39$, $p < 0.01$) after ulceration. Besides, as seen on Fig. 2, low-level laser therapy significantly increased the activities of complexes II ($F(10) = 4.08$, $p < 0.01$) and IV ($F(10) = 5.21$, $p < 0.01$) but did not affect succinate dehydrogenase activity ($F(10) = 0.06$, $p = 0.63$).

4. Discussion

Wound healing has three phases, where a substrate is laid down, then cells proliferate, and then there is remodeling of tissue. Evidence from literature suggest that laser biostimulation produces its primary effect during the cell proliferation phase of the wound healing process. At cellular level, photo-irradiation at low power causes significant biological effects such as cellular proliferation, collagen synthesis, the release of growth factors from cells [17] and macrophage and lymphocyte stimulation [18]. Laser photobiomodulation has also been shown to alter the expression

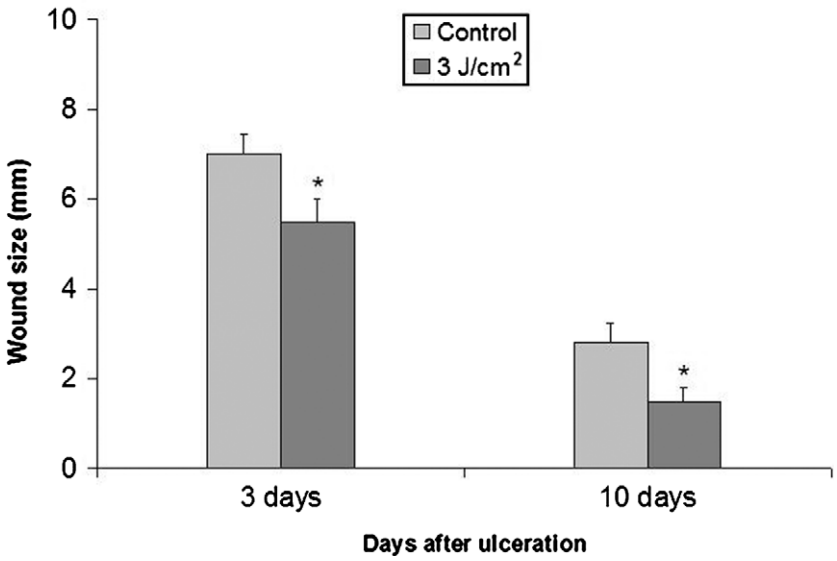

Fig. 1. Effect of low-level laser therapy on wound healing (wound size) 3 and 10 days after ulceration. On the medial dorsal portion a circular area of skin (approximately 8 mm of diameter) was removed with a punch. In low-level laser therapy group, wounds were treated immediately after they were made and every day after with laser for 10 days. The laser irradiation was performed in five distinct regions around the wound margin. * Different from control, $p < 0.01$ (Student's t-test).

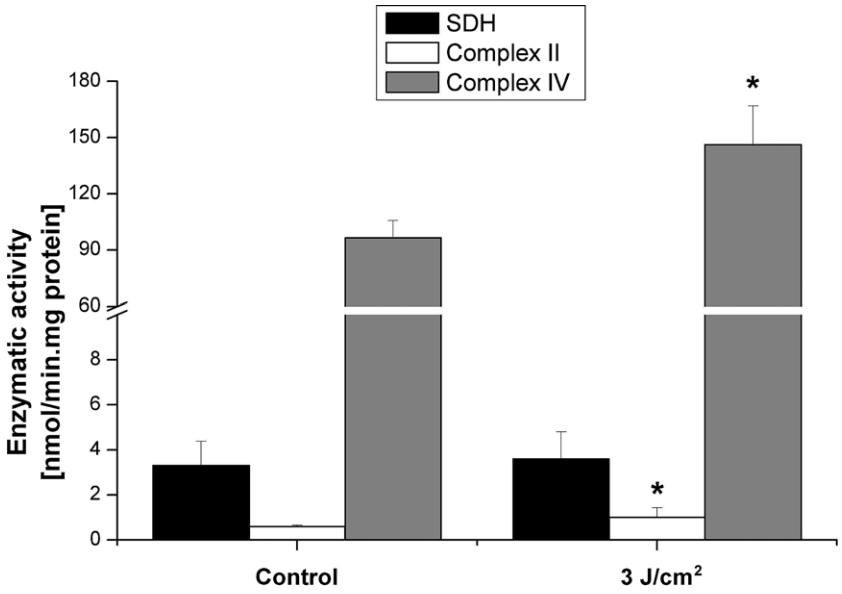

Fig. 2. Effect of low-level laser therapy on mitochondrial respiratory chain complexes II and IV and succinate dehydrogenase (SDH) activity. On the medial dorsal portion a circular area of skin (approximately 8 mm of diameter) was removed with a punch. In low-level laser therapy group, wounds were treated immediately after they were made and every day after with laser for 10 days. The laser irradiation was performed in five distinct regions around the wound margin. * Different from control, $p < 0.01$ (Student's t-test).

of genes involved in wound healing and possibly pain modulation [19]. In this context, low-level laser therapy has been shown to accelerate wound healing [9].

The results obtained in our work showed that low-level laser therapy improved wound healing. The wound size was significantly smaller 3 and 10 days after ulceration (Fig. 1). Besides, our results also showed a significant increase in complexes II and IV activities in wound after low-level laser therapy, but not in succinate dehydrogenase activity (Fig. 2). The low-level laser used in this study was a arsenium–gallium (AsGa) with a wavelength of 904 nm. Other studies, however, emphasize that depending on the applied dose, wavelength, irradiation time and also the conditions of the treated tissue, different biological answers can be achieved [20–23].

In this context, our findings may be in accordance to another study, where Karu and colleagues (1995) [10] described that near infrared light (He–Ne laser with 633 nm) increased ATP level in cells, since complexes II and IV activities were increased. Other studies also showed

that irradiation with light at wavelengths of 415, 602, 633, 650 and 725 nm enhances ATP synthesis [20–23]. However, it was already demonstrated that lights at wavelengths of 477, 511 and 554 nm do not influence the rate of this process [21].

Data from literature strongly suggest that cytochrome *c* oxidase (mitochondrial respiratory chain complex IV) is a key photoacceptor of light in the near infrared spectral range [10,12,24]. We speculate that this enzyme could act in a similar way in the wavelength used in our work (904 nm). Besides, it was already demonstrated that 660 to 680 nm irradiation increased electron transfer in purified cytochrome *c* oxidase [25], mitochondrial respiration and ATP synthesis in isolated mitochondria [23] and upregulated cytochrome *c* oxidase activity in cultured neuronal cells [12]. It is also known that near-infrared light therapy results in initiation of a mitochondrial signaling cascade which promotes cellular proliferation and cytoprotecton at cellular level [10–13].

This work confirms other data from literature, where cytochrome *c* oxidase seems to be activated by low-level laser therapy. However, we also showed, for the first time, that complex II activity was also activated and that succinate dehydrogenase was not altered by such treatment. Further studies are being carried out in order to investigate the effect of low-level laser therapy on activities of the Krebs cycle enzymes. Besides, more studies aim to evaluate mitochondrial enzymes activities after different doses and irradiation time of low-level laser.

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