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

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ABSTRACT

Background: Recent studies demonstrate that low-level laser therapy (LLLT) modulates many biochemical processes, especially the decrease of muscle injures, the increase in mitochondrial respiration and ATP synthesis for accelerating the healing process.
Objective: In this work, we evaluated mitochondrial respiratory chain complexes I, II, III and IV and succinate dehydrogenase activities after traumatic muscular injury.
Methods: Male Wistar rats were randomly divided into three groups ($n = 6$): sham (uninjured muscle), muscle injury without treatment, muscle injury with LLLT (AsGa) 5 J/cm^2. Gastrocnemius injury was induced by a single blunt-impact trauma. LLLT was used 2, 12, 24, 48, 72, 96, and 120 hours after muscle-trauma.
Results: Our results showed that the activities of complex II and succinate dehydrogenase after 5 days of muscular lesion were significantly increased when compared to the control group. Moreover, our results showed that LLLT significantly increased the activities of complexes I, II, III, IV and succinate dehydrogenase, when compared to the group of injured muscle without treatment.
Conclusion: These results suggest that the treatment with low-level laser may induce an increase in ATP synthesis, and that this may accelerate the muscle healing process.

1. Introduction

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

LLLT has been used to treat muscle pain, although the biological mechanisms of the beneficial results observed in clinical trials remain unclear. The ability of LLLT to reduce the duration of acute inflammation and accelerate tissue repair in tendon and muscle injuries was proposed [5–7]. Lubart et al. [8] suggested that LLLT might promote changes in the cellular redox state, playing a pivotal role in sustaining cellular activities, and promoting photobiostimulative processes.

Other studies, however, emphasize that depending on the applied dose, wavelength, irradiation time, and the conditions of the treated tissue, different biological answers can be achieved [9–12].

The aim of this work was to evaluate the activities of mitochondrial respiratory chain complexes I, II, III and IV, and of succinate dehydrogenase in traumatic muscle injury after irradiation with LLLT.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–300 g) obtained from the Central Animal House of the Universidade do Extremo Sul Catarinense, Santa Catarina, Brazil, were caged in groups of five, provided with commercial rat chow and water ad libitum, and maintained on a 12-h light/12-h dark cycle. The animals were randomly divided into three groups ($n = 6$): sham (uninjured muscle), muscle injury without treatment, and muscle injury with LLLT (AsGa) 5 J/cm^2. All studies were performed in accordance with the National Institutes of Health guidelines and with the approval of the Ethics Committee of the Universidade do Extremo Sul Catarinense, Santa Catarina, Brazil.

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2.2. Muscle injury model

The muscle-trauma model was described by Rizzi et al. [13]. The animals were anesthetized with an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (15 mg/kg). Gastrocnemius injury was induced by a single blunt-impact trauma in a press developed by the Centro Industrial de Equipamentos de Ensino e Pesquisa (CIDEP/RS, Brazil). Briefly, injury was produced by a metal mass (0.459 kg) falling through a metal guide from a height of 18 cm. The impact kinetic energy delivered was 0.811 J. Control rats were also anesthetized to ensure standardization, but without muscle-trauma.

2.3. Treatment

The low-level laser used in this study was an arsenium–gallium (AsGa) laser with a wavelength of 904 nm, and power ranging from 15 to 30 mW. The total dose per session was 5 J/cm^2. Laser irradiation was performed over five distinct regions around the damaged area, with the laser pen being kept perpendicular to the injury at a distance of 1 cm per point, as described by Morrone [14]. The animals were irradiated 2, 12, 24, 48, 72, 96, and 120 h after the trauma.

2.4. Sample preparation

Two hours after the last application, the animals were killed by decapitation and the blood was collected. The medial portion of gastrocnemius was surgically removed on the impact area and immediately processed, aliquoted and stored at −70 °C for later analysis. The gastrocnemius was homogenized in the buffer used for each technique. The homogenates were centrifuged at 1000 *g* for 10 min at 4 °C, and the supernatants were kept at −70 °C until being used for the experiments. The maximal period between homogenate preparation and biochemical analysis was always less than 5 days.

2.5. Biochemical assays

2.5.1. Activities of the mitochondrial respiratory chain enzymes

The gastrocnemius was homogenized (1:10, w/v) in SETH buffer (250 mM sucrose, 2 mM EDTA, 10 mM Trizma base, 50 IU/ml heparin, pH 7.4). The homogenates were centrifuged at 800 *g* for 10 min and the supernatants were used for determining the activities of the mitochondrial respiratory chain enzymes (complexes I, II, III and IV). On the day of the assays, the samples were frozen and thawed thrice in hypotonic assay buffer to fully expose the enzymes to substrates and achieve maximal activities. NADH (nicotinamide adenine dinucleotide) dehydrogenase (complex I) was evaluated according to the method described by Cassina and Radi [15] by the rate of NADH-dependent ferricyanide reduction at 420 nm. The activities of succinate (DCIP oxidoreductase – complex II) and succinate cytochrome *c* oxidoreductase (complex II–III) were determined according to the method of Fischer et al. [16]. Complex II activity was measured by following the decrease in absorbance due to the reduction of 2,6-DCIP at 600 nm. Complex II–III activity was measured by cytochrome *c* reduction from succinate. The activity of cytochrome *c* oxidase (complex IV) was assayed according to the method described by Rustin et al. [17], measured by following the decrease in absorbance due to the oxidation of previously reduced cytochrome *c* at 550 nm. The activities of the mitochondrial respiratory chain complexes were expressed as nmol/min/mg protein.

2.5.2. Protein determination

The amount of protein in the samples tested for activities of mitochondrial respiratory chain enzymes was determined using the Lowry [18] technique.

2.6. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by LSD test when p values were significant ($p < 0.05$). All analyzes were performed using the Statistical Package for the Social Science (SPSS) software.

3. Results

In this work, we measured mitochondrial respiratory chain complexes I, II, III and IV and succinate dehydrogenase activities in injured muscle after treatment with low-level laser for 5 days. We verified that the activities of complex II and succinate dehydrogenase after 5 days of muscular lesion were significantly increased when compared to the control group. Moreover, our results showed that LLLT significantly increased the activities of complexes I, II, III and IV and of succinate dehydrogenase, when compared to the injured muscle without treatment group (Figs. 1 and 2).

4. Discussion

The inflammatory response to direct trauma as well as stretch injury consists of neutrophilia, neutrophil activation, and of accumulation of neutrophils within the injured muscle as early as 1–2 h. In this early inflammatory stage, cellular debris is removed by the infiltrating neutrophils and is followed by a regenerative response during which satellite cells proliferate to replace the previously damaged and phagocytosed muscle [19].

Skeletal muscle healing has three phases, where a substrate is laid down, then cells proliferate, and then there is a remodeling of the tissue. Evidence from literature suggests that laser biostimulation produces its primary effect during the cell proliferation phase of the muscular 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 and macrophage and lymphocyte stimulation [20,21].

The results obtained in our work showed a significant increase in complexes I, II, III and IV activities, and succinate dehydrogenase activity in the injured muscle after LLLT. The LLLT used in this study was an arsenium–gallium (AsGa) laser with a wavelength of 904 nm.

Several lines of evidence show that mitochondria are sensitive to irradiation with monochromatic visible light. The illumination of isolated rat liver mitochondria increases adenosine triphosphate (ATP) synthesis and the consumption of O_2. Irradiation with light at 904 nm increases the mitochondrial membrane potential and proton gradient, causes changes in mitochondrial optical properties, modifies some NADH-linked dehydrogenase reactions, and increases the rate of ADP/ATP exchange (ADP, adenosine diphosphate) [9].

Increased activity of mitochondrial electron transport can be associated with a variety of mitochondrial enzyme activities. Photostimulatory effects on a variety of mitochondrial enzymes (protein complexes in respiratory transport chain) have been proposed and studied by different researchers. However, most of the proposed mechanisms are based on oxygen consumption studies and lack direct experimental support [9,22,23].

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. We speculate that this enzyme could act in a similar way in the wavelength used in our work (904 nm) [11,23].

Moreover, it was already demonstrated that 660–680 nm irradiation increased electron transfer in purified cytochrome *c* oxidase,

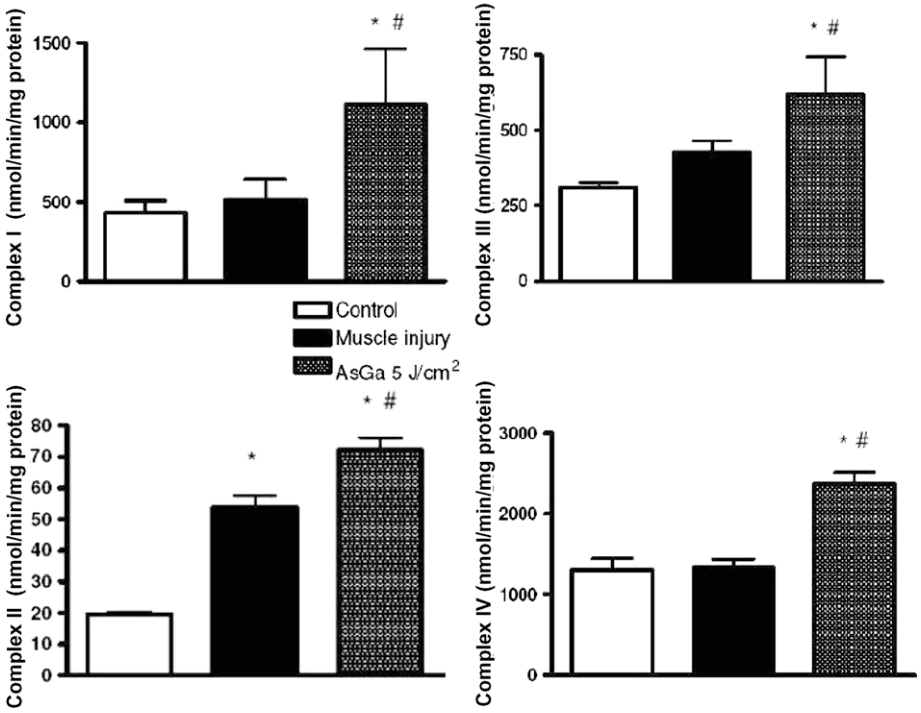

Fig. 1. Effect of low-level laser therapy on mitochondrial respiratory chain complexes I, II, III and IV activity in skeletal muscle after injury (5 days). Data are expressed as mean ± SEM for six animals. Different from control ($^{*}p < 0.05$) and different from muscle injury without treatment ($^{\#}p < 0.05$) (LSD test).

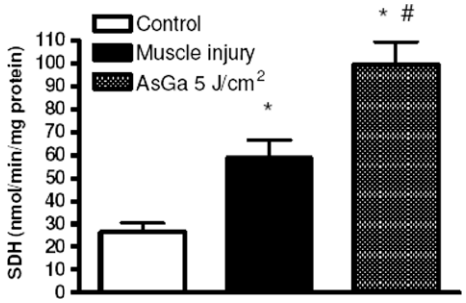

Fig. 2. Effect of low-level laser therapy on succinate dehydrogenase activity in skeletal muscle after injury (5 days). Data are expressed as mean ± SEM for six animals. Different from control ($^{*}p < 0.05$) and different from muscle injury without treatment ($^{\#}p < 0.05$) (LSD test).

mitochondrial respiration and ATP synthesis in isolated mitochondria and upregulated cytochrome *c* oxidase activity in cultured neuronal cells. It is also known that near-infrared light therapy results in initiation of a mitochondrial signaling cascade that promotes cellular proliferation and cytoprotection at cellular level [9,12].

When photons from laser light energize the metal sites in these complexes, shaking/vibration of these metals alter either the enzyme conformation or the redox reaction, and this in turn increases the transfer of electrons throughout the respiratory chain and/or pumping of protons across the inner mitochondrial membrane. Increased transference of electrons and protons accelerates oxidative metabolism and leads to in increase in ATP synthesis. Increased ATP production may in turn promote cellular metabolism. We speculate that the increase in ATP production due to LLLT may induce acceleration in muscle healing process, especially in the inflammatory phase [22,24,25].

5. Conclusion

The present findings showed that LLLT increased the activity of mitochondrial respiratory chain complexes I, II, III and IV, and of succinate dehydrogenase. These results suggest that the treatment with low-level laser may induce an increase in ATP synthesis, and that this may accelerate the muscle healing process. Although this study has been conducted with an animal model, it is possible to extrapolate our results to the clinical practice with humans, suggesting the positive effect of laser on muscle injury. However, studies with humans are necessary to reinforce our findings.

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References

[1] J.S. Carrillo, J. Calatayud, F.J. Manso, E. Barberia, J.M. Martinez, M. Donado, A randomized double-blind clinical trial on the effectiveness of helium–neon laser in the prevention of pain, swelling and trismus after removal of impacted third molars, Int. Dent. J. 40 (1990) 31–36.
[2] L. Lilge, K. Tierney, E. Nussbaum, Low-level laser therapy for wound healing: feasibility of wound dressing transillumination, J. Clin. Laser Med. Surg. 18 (2000) 235–240.
[3] Y. Maegawa, T. Itoh, T. Hosokawa, K. Yaegashi, M. Nishi, Effects of near-infrared low-level laser irradiation on microcirculation, Lasers Surg. Med. 27 (2000) 427–437.
[4] A. Schlager, P. Kronberger, F. Petschke, H. Ulmer, Low-power laser light in the healing of burns: a comparison between two different wavelengths (635 nm and 690 nm) and a placebo group, Lasers Surg. Med. 27 (2000) 39–42.
[5] D. Hawkins, N. Houreld, H. Abrahamse, Low level laser therapy (LLLT) as an effective therapeutic modality for delayed wound healing, Ann. NY Acad. Sci. 1056 (2005) 486–493.
[6] R.T. Chow, L. Barnsley, Systematic review of the literature of low level laser therapy (LLLT) in the management of neck pain, Lasers Surg. Med. 37 (2005) 46–52.
[7] E.S. Pessoa, R.M. Melhado, L.H. Theodoro, V.G. Garcia, A histologic assessment of the influence of low-intensity laser therapy on wound healing in steroid-treated animals, Photomed. Laser Surg. 22 (2004) 199–204.

[8] R. Lubart, M. Eichler, R. Lavi, H. Friedman, A. Shainberg, Low energy laser irradiation promotes cellular redox activity, Photomed. Laser Surg. 23 (2005) 3–9.
[9] T. KARU, Mechanisms of interaction of monochromatic visible light with cells, In: Proceedings of Effects of Low Power Light on Biological Systems, vol. 26, 1995, pp. 2–9.
[10] M.T. Wong-Riley, X. Bai, E. Buchmann, H.T. Whelan, Light emitting diode treatment reverses the effect of TTX on cytochrome oxidase in neurons, NeuroReport 12 (2001) 3033–3037.
[11] M.T. Wong-Riley, J. VerHoeve, M. Henry, E.V. Buchman, Mitochondrial signal transduction in accelerated wound and retinal healing by near-infrared light therapy, Mitochondrion 4 (2004) 559–567.
[12] C.F. Rizzi, J.L. Mauriz, D.S.F. Corrêa, Effects of low-level laser therapy (LLLT) on the nuclear factor (NF)-κB signaling pathway in traumatized muscle, Lasers Surg. Med. 38 (2006) 704–713.
[13] E.T. Morrone, Muscular trauma treated with a Ga–Al–As diode laser: in vivo experimental study, Lasers Med. Sci. 13 (1998) 293–298.
[14] A. Cassina, R. Radi, Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport, Arch. Biochem. Biophys. 328 (1996) 309–316.
[15] J.C. Fischer, W. Ruitenbeek, J.A. Berden, J.M. Trijbels, J.H. Veerkamp, A.M. Stadhouders, R.C. Sengers, A.J. Janssen, Differential investigation of the capacity of succinate oxidation in human skeletal muscle, Clin. Chim. Acta 153 (1985) 23–26.
[16] P. Rustin, D. Chretien, T. Bourgeron, B. Gerard, A.R. Saudubray, Biochemical and molecular investigations in respiratory chain deficiencies, Clin. Chim. Acta 228 (1994) 35–51.
[17] O.H. Lowry, N.G. Rosebough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[18] H. Toumi, T.M. Best, The inflammatory response: friend or enemy for muscle injury? Br. J. Sports Med. 37 (2003) 284–286.
[19] A.P. Sommer, A.L.B. Pinheiro, A.R. Mester, R.P. Franke, H.T. Whelan, Biostimulatory windows in low intensity laser activation: lasers, scanners and NASA's light-emitting diode array system, J. Clin. Laser Med. Surg. 19 (2001) 29–34.
[20] T.P. O'Brien, Q. Li, M.F. Ashraf, D.M. Matteson, W.J. Stark, C.C. Chan, Inflammatory response in the early stages of wound healing after excimer laser keratectomy, Arch. Ophthalmol. 116 (1998) 1470–1474.
[21] W. Yu, J.O. Naim, M. Mcgowan, Photomodulation of oxidative metabolism and electron chain enzymes in rat liver mitochondria, Photochem. Photobiol. 66 (1997) 866–871.
[22] D. Pastore, M. Greco, S. Passarella, Specific helium–neon laser sensitivity of the purified cytochrome *c* oxidase, Int. J. Radiat. Biol. 6 (2000) 863–870.
[23] P.C.L. Silveira, E.L. Streck, R.A. Pinho, Evaluation of mitochondrial respiratory chain activity in wound healing by low-level laser therapy, Photochem. Photobiol. 86 (2007) 279–282.
[24] S. Hallen, P. Brzezinski, Light-induced structural changes in cytochrome *c* oxidase: implication for the mechanism of electron and proton gating, Biochim. Biophys. Acta 1184 (1994) 207–218.
[25] N. Grossman, N. Schneid, 780 nm low power diode laser irradiation stimulates proliferation of keratinocyte cultures: involvement of reactive oxygen species, Lasers Surg. Med. 22 (1998) 212–218.