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BRIEF REPORT

Effect of photobiomodulation on expression of IL-1β in skeletal muscle following acute injury

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Abstract Muscle repair is regulated by growth factors and cytokines. Low-level laser therapy (LLLT) seems to influence acute inflammation and accelerate skeletal muscle repair. This study verifies the effect of LLLT on the expression of IL-1β in the tibialis anterior (TA) muscle of rats following acute injury. Wistar rats (n=35) were allocated into three groups: control (without lesion and LLLT, n=5), injury group (n=15), and injury + LLLT group (n=15). The acute injury was induced by the contact with a cooled metal probe (3 mm in diameter) during 10 s, twice, in the same muscle area. LLLT was used three times a week using the InGaAlP laser (660 nm; beam spot of 0.04 cm^2, output power of 20 mW, power density of 500 mW/cm^2, and energy density of 5 J/cm^2 during 10 s). The animals were analyzed at 1, 7, and 14 days following injury. TA muscles samples were used for obtaining total RNA and performing cDNA synthesis. Real-time polymerase chain reactions were realized using IL-1β primer. There was a decrease in IL-1β expression after 7 days in LLLT group in comparison with the no treated group. In conclusion, LLLT was able to decrease IL-1β expression during the skeletal muscle repair following an acute injury.

Keywords IL-1β · LLLT · Skeletal muscle · Repair

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Introduction

Recent studies on musculoskeletal disorders link local and systemic inflammation to myopathic changes that are accompanied by pain and a decline in function [1]. Following an injury, inflammatory cells reach the muscle tissue and persist there during the repair and regeneration process [2]. This initial cell infiltration includes both neutrophils and macrophages and is associated with an increase in pro-inflammatory cytokines, such as TNFα, IL-1β, and IL-6 [2, 3].

IL-1 is mainly produced by macrophages, monocytes, and dendritic cells. This cytokine has two distinct subtypes, one (IL-1α) that stays primarily in the cytosol of cells and a second one (IL-1β) that becomes active upon cleavage by a protease produced in monocytes [4]. There are many conditions associated with an increase in IL-1 including modulation of inflammation, fever, and cell proliferation. These

degenerative effects are more intense when IL-1 acts synergistically with TNF-α to promote the catabolism of lean tissue [5]. IL-1β is also released by injured muscle cells to attract inflammatory cells such as neutrophils and macrophages [3] and plays a critical role in both host defense and muscle tissue remodeling following injury.

Low-level laser therapy (LLLT) has demonstrated to induce improvement on inflammatory and repair process in skeletal muscles [6–9]. However, a number of protocols and different LLLT parameters have been used and the results regarding the modulation of cytokine production are different [6, 8]. Therefore, the aim of the study was verify the influence of LLLT on the mRNA of IL-1β in tibialis anterior (TA) muscle rats following cryoinjury.

Methods

The experiments were carried out in accordance with the guidelines of the National Council for the Control of Animal Experimentation and Universidade Nove de Julho Ethical Committee on Animal Research (approval 13/2007).

Male Wistar rats (body weight 234±37.99 g) were maintained under controlled conditions as previously described [6, 11] and allocated into three groups: control (n=5, without injury and untreated), injury group (n=15), and injury + LLLT group (n=15). Animals from the control group were analyzed at 1 day and injury animals were analyzed at 1, 7, and 14 days following injury. TA muscle was submitted to a cryoinjury as previously described [6, 10, 11]. Animals were anesthetized with 1 ml/kg of 1 % ketamine and 2 % xylazine. The TA muscle was exposed by incision and submitted to cryoinjury by the contact with a cooled metal probe (3 mm in diameter) during 10 s, twice, in the same muscle area. The left TA muscle was injured and the right side was used as control. After cryoinjury, the wounds were sutured.

LLLT procedure

The LLLT equipment used was the InGaAlP diode laser (MMOptics Ltd., São Carlos, SP, Brazil) of 660 nm, a beam spot of 0.04 cm^2, an output power of 20 mW, an energy density of 5 J/cm^2, and a power density of 500 mW/cm^2 for 10 s. The laser beam was placed in contact with the skin surface corresponding to the cryolesioned area and the irradiation was applied at eight points around the cryolesion area as previously described [6, 11].

The total energy used per treatment was 1.6 J, that is, eight application points receiving 0.2 J each. It used a LaserCheck power meter (Coherent, Santa Clara, CA, USA) to verify the output power of the laser device used.

The LLLT was applied three times a week, starting 24 h after injury, with a 24-h interval between sessions. Groups at 1, 7, and 14 days received one, three, and six sessions, respectively. At the end of the treatment, animals were killed and TA muscles were quickly dissected and frozen in liquid nitrogen.

Total RNA was isolated from TA muscles using cold Trizol Reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. cDNA synthesis was performed using AMV reverse transcriptase (Invitrogen, Brazil) and all samples received treatment with DNase (Invitrogen) to avoid DNA contamination as previously described [6, 11].

Real-time PCR was performed using a 7500 Sequence Detection System (ABI Prism, Applied Biosystems, Foster City, CA) and the SYBRGreen kit (Applied Biosystems, USA). Specific primers for rat IL-1β (forward 5′-CACCTCTCAAGCAGAGCACAG-3′; reverse 5′-GGGTTCCATGGTGAAGTCAAC-3′ (GenBank accession # M98820)) were used for this procedure and a constitutive gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was used to normalize the data using the same amount of cDNA. GAPDH primers were forward 5′-TGCACCAC CAACTGCTTAGC-3′ and reverse GCCCCACGGCCATCA-3 (GenBank accession # NM 017008).

To normalize the data for the control, injury, and injury + LLLT, arbitrary units were calculated by subtracting the average Ct (threshold cycle) value of IL-1β mRNA from the average Ct value of the GAPDH as described following: arbitrary unit = $2^{-\Delta\Delta CT}$ and ΔΔTC = IL-1β ΔCt−GAPDH ΔCt.

IL-1β mRNA data are presented as mean values and standard deviation. The studied data follow a Gaussian (normal) distribution, as verified by Levene's test. In this manner, parametric methods were used to detect differences between all possible pairs in the within-group- and within-day analysis. The program GraphPad Prism 4.0 statistical software (GraphPad Software, San Diego, CA, USA) was used for statistical analysis. Statistical significance was assessed by one-way ANOVA and the Tukey test, with the level of significance of $P<0.05$.

Results

The results of the mRNA IL-1β are presented in Fig. 1. After 1 day following injury, LLLT induced a decrease in IL-1β mRNA expression in comparison to non-LLLT injury groups, although not significantly. There were also no significant differences between experimental groups and the control group.

After 7 days, it noted a significant decrease in IL-1β mRNA expression in the injury + LLLT group in comparison to the injury group without laser treatment. In the same period, there was also a significant increase in IL-1β mRNA in the injury group in comparison to all other groups in all periods, except the injury group at 14 days. At 14 days after

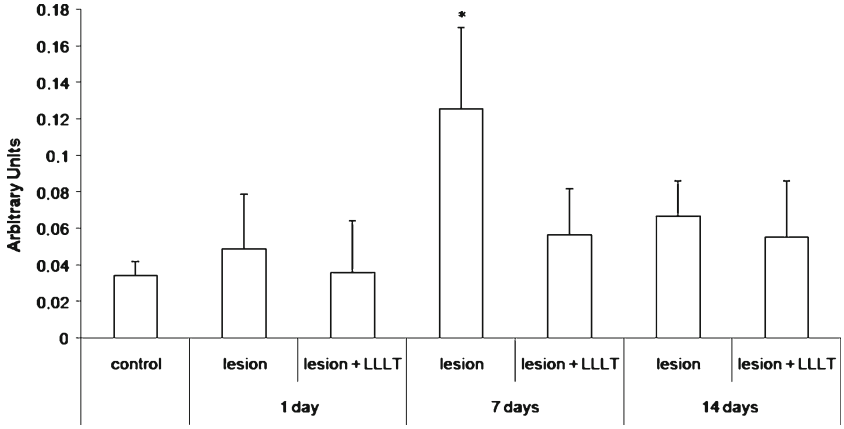

Fig. 1 Results of the mRNA IL-1β

injury, there was no significant difference in IL-1β mRNA expression, when comparing LLLT injury and non-LLLT injury groups, or between these groups and the control group.

Discussion

The results of the present study demonstrate that, when using the cryoinjury model, there was a significant increase in IL-1β mRNA in the non-treated injury group 7 days following injury and that LLLT (660 nm, 20 mW, and 5 J/cm^2) was able to promote a significant decrease in this expression in the same period. No statistically significant differences between the LLLT and non-LLLT groups were found for the other periods.

There are very few papers on the relationship between LLLT and IL-1β modulation in skeletal muscle following injury. Albertini et al. [8] demonstrated that the mRNA expression of TNF-α, IL-1β, and IL-6 was increased 3 h after carrageenan injection in subplantar muscle tissue of rat paws. LLLT at wavelengths of 660 or 684 nm (30 mW, 7.5 J/cm^2) induced a decrease in TNF-α, IL-1β, and IL-6 mRNA expression 3 h after irradiation. In the present study, a nonsignificant increase in IL-1β expression was observed after 1 day in the injury group in comparison to the control group and a nonsignificant decrease was observed in the injury + LLLT group in comparison to the group submitted to injury alone.

These differences in time and significance may be related to differences in the laser parameters and/or the type of injury. Carrageenan subplantar injection induces a biphasic cellular infiltrate. The first peak was seen at 3 h and the delayed peak 48 h after injection. In the early peak, the cellular infiltrate is mainly made up of neutrophils, whereas the infiltrate in the delayed peak is composed of macrophages, eosinophils, and lymphocytes [12–14]. Four hours after injection, carrageenan induced a considerable increase in the expression of IL-1β in the edema fluid of the paw [13].

Neutrophils are the first to respond in acute muscle injury. Typically, a peak in concentration is seen between 6 and 24 h after injury, with a rapid decline [2]. Macrophages reach significantly elevated concentrations at about 24 h post-injury, continuing to increase in numbers until reaching peak concentrations in the muscle at about 4 days, remaining significantly elevated for many days afterward [2]. In the muscle injury model employed in the present study, peak IL-1β RNA expression took place after 7 days, likely produced by macrophages mainly, and LLLT was able to significantly reduce this expression.

In a previous study by our research group using the same irradiation parameters as those employed in the present study, LLLT was found to cause a significant decrease in TNF-α mRNA expression at 1 and 7 days following injury in comparison to the control group, which could explain the decrease in IL-1β presented in this study [6].

Although TNF-α plays a pivotal role in regulating the expression of IL-1β as it is upstream in this cytokine cascade, and has the ability to upregulate IL-1β production [8], it is important to consider that an increase/decrease in gene expression is not always accompanied by a corresponding increase/decrease in protein production as it may be under post-transcriptional or post-translational regulation.

The anti-inflammatory effects of LLLT on muscle tissue have previously been suggested [6, 8, 11, 15]. Accordingly, the present study demonstrates that LLLT was able to modulate IL-1β during muscle repair following an acute injury.

Pro-inflammatory cytokines, such as TNF-α and IL-1β, cause the translocation of NFκβ to the cell nucleus [16, 17]. Once in the nucleus, NF-κB can induce the transcription of iNOS, TNF-α, and IL-1, which may then promote further NF-κB activation, as well as an increase in the expression of other inflammatory mediators, such as IL-6, thereby perpetuating the inflammatory process [16, 17]. Moreover, the activation of NF-κB participates in a key signaling pathway resulting in the inhibition of myogenic differentiation [18–21].

In addition, IL-1β inhibits myoblast differentiation by reducing the IGF-1 ability to promote an increase in the

synthesis of myogenin, an important myogenic regulatory factor involved in cell muscle differentiation, and subsequently in increasing myosin expression [22].

It is noteworthy that this study evaluated the effect of LLLT only on gene expression of IL-β. Additional studies involving protein analysis and other experimental periods could complement the data presented.

In conclusion, the present study shows that LLLT was able to decrease IL-1β mRNA expression 7 days after cryoinjury in TA muscle rats, thus favoring the modulation of the inflammatory process, and possibly preventing the inhibition of myogenic differentiation caused by elevated IL-1β concentrations.

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