Antinociceptive Effects of Low-Level Laser Therapy at 3 and 8 j/cm^2 in a Rat Model of Postoperative Pain: Possible Role of Endogenous Opioids

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Low-level laser therapy (LLLT) is the direct application of light to stimulate cell responses (photobiomodulation) to promote tissue healing, reduce inflammation, and induce analgesia; the molecular basis for these effects of LLLT remains unclear. The objective of this study was to evaluate the analgesic effect of LLLT in the rat plantar incision model of postoperative pain as well as to investigate some of the possible mechanisms involved in this effect. Wistar rats were submitted to plantar incision and treated with LLLT (830 nm, continuous-mode, 30 mW/cm^2, 1–12 J/cm^2). Postoperative thermal and mechanical hypersensitivity were monitored for 24 hours post-incision. In addition, the animals were pretreated with saline, naloxone (a nonselective opioid receptor antagonist; 20 μg/5 μl) or methysergide (5-HT$_{2C}$, 5-HT$_{2A}$, 5-HT$_7$, 5-HT$_{5a}$, 5-HT$_6$, and 5-HT$_{1F}$ receptors antagonist; 30 μg/5 μl). Moreover, 24 hours after incision and treatment, the TNF-α and IL-1β levels in serum were evaluated. Our results demonstrate, for the first time, that LLLT at 3 or 8 J/cm^2, but not at 1–2, 4–7, or 9–12 J/cm^2, induced an analgesic effect on postoperative pain. Naloxone, but not methysergide, blocked the LLLT-induced anti-nociceptive effect. Additionally, IL-1-β and TNF-α production significantly decreased after LLLT at 3 or 8 J/cm^2. Our results suggest that LLLT at 3 or 8 J/cm^2 primarily modulates the endogenous opioids system and is not directly mediated by serotonergic receptors. Reduction of IL-1β and TNF-α may play a role in the antinociceptive action of LLLT. Lasers Surg. Med. 49:844–851, 2017. © 2017 Wiley Periodicals, Inc.

Key words: low-level laser therapy; postoperative pain; endogenous opioids

INTRODUCTION

Postoperative pain results from noxious surgical stimulation of the skin, subcutaneous tissues, viscera, and neural structures leading to a reduction in the threshold of afferent nerve endings; moreover, postoperative pain affects one in every two patients undergoing surgery [1]. Despite advances in understanding the mechanisms of postoperative pain, variations in treatment regimens have made pain management challenging [2]. Even in the very early postoperative period hours after incision, sensory afferent neurons exhibit spontaneous and stimulus-evoked activity that mediates hyperalgesia and allodynia [3].

Inflammatory mediators released at the site of tissue injury induce a reduction in the thermal and mechanical thresholds leading to pain hypersensitivity [4–6], and studies have provided evidence for the role of cytokines in the induction and maintenance of postoperative pain [7]. Furthermore, the levels of tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) are increased two hours after surgery. Additionally, IL-6 and IL-1β are also up-regulated in the dorsal root ganglion at 14 and 28 days after surgery [8].

Several drugs such as non-steroidal anti-inflammatory drugs or opioids are commonly used for the treatment of relief of pain. However, they may have serious side effects including: gastrointestinal and physical effects, dependence, and tolerance [9]. In this context, studies of non-pharmacological therapies for the treatment pain and chronic inflammation could be beneficial.

Low-level laser therapy (LLLT), also known as photobiomodulation, is a low intensity light therapy that is commonly performed with a low powered laser or LED typically in the 10–500 mW power range [10,11]. Light with a wavelength in the red to near infrared region of the spectrum (600–1100 nm), is generally employed because these wavelengths can penetrate skin, and soft/hard tissues and promote tissue regeneration, reducing the inflammation, and relieving pain. These effects are

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

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Accepted 2 June 2017
Published online 3 July 2017 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/lsm.22696

photochemical instead of thermal [10]. The photobiomodulation effects evoked by LLLT are related to increases in the adenosine triphosphate (ATP) levels, redox reactions, and oxygen exchange [12]. As a result of these alterations, other secondary effects are induced, such as pain reduction [13,14], acceleration of healing [15], vasodilation, and oedema reduction [16,17].

In many studies, LLLT is used in the treatment of pain with laser highlighting at 830 nm [18–20]. However, prior studies are controversial due to the variation in the parameters for the use of irradiation, particularly with energy densities ranging from 1 to 15 J/cm^2, wavelengths from 780 to 905 nm and power from 30–450 mW, leading to a lack of consensus on the ideal parameters for laser therapy [20]. Therefore, despite the increasing knowledge of LLLT in pain management, it is necessary to evaluate the acute effects in postoperative pain.

In this study, we evaluated the acute effect of LLLT in the thermal and mechanical hypersensitivity produced by plantar incision in the rat. We also investigated the possible role of (i) the opioidergic and serotoninergic systems; and (ii) the involvement of pro-inflammatory cytokines (TNF-α and IL-1β) in the acute effect mediated by LLLT.

MATERIALS AND METHODS

Animals

The experiments were conducted using male Wistar rats (Rattus norvegicus) (aged 9 weeks and weighing $\pm$ 200 g) from the main animal house of the Federal University of Alfenas-UNIFAL. Animals were housed at a controlled temperature ($24 \pm 1^{\circ}$C) and on a 12-hour light-dark cycle (dark cycle beginning at 7 am), and they had free access to food and water. The guidelines of the Committee for Research and Ethical Issues of IASP were followed throughout the experiments. All experimental protocols were performed after approval by the Committee of Animal Experimentation of the UNIFAL, protocol 622/2015.

Plantar Incision Model of Postoperative Pain

Plantar incision was performed as previously described [21]. Briefly, rats were anaesthetized with isoflurane (3% isoflurane mixed with 100% oxygen at a flow rate of 5 L minute^{-1}) and the plantar surface of the right hind paw was sterilely prepared. A 1 −cm longitudinal incision was cut with a number 11 scalpel through the skin and fascia of the plantar aspect of the paw, starting 0.5 cm from the proximal end of the heel and extending toward the toes. The plantaris muscle was elevated and longitudinally incised. After the bleeding was stopped through gentle pressure, the skin was opposed with two single sutures using 5–0 nylon. The animals could recover in their home cages.

Intrathecal Injections

The injections were performed in rats anesthetized with isoflurane (3%). A 1-inch, 25-G needle was transcutaneously introduced at the L5-L6 level into the subarachnoid space [22,23]. A sudden lateral movement of the tail was taken as indicative that the needle entered the subarachnoidal space. A constant 5 μl volume was injected, and the syringe was then held in position for a few seconds and gradually removed to avoid any durg outflow. Saline, naloxone hydrochloride (opioid receptors antagonist; 20 μg/5 μl, Sigma, St. Louis, MO) or methysergide maleate (5-HT$_{2C,}$ 5-HT$_{2A}$, 5-HT$_7$, 5-ht$_{5a}$, 5-HT$_{6,}$ and 5-HT$_{1F}$ receptors antagonist; 30 μg/5 μl, Sigma, St. Louis, MO) dissolved in saline was intrathecally injected 15 minutes before LLLT.

Paw Mechanical Sensitivity

Mechanical sensitivity was measured using an electronic von Frey device (Insight Equipamentos, Ribeirão Preto, SP, Brazil). Rats were placed in a wire chamber where they remained until exhibiting brief exploratory behavior (~15 minutes). The electronic pressure transducer contacted the hind paw through a disposable polypropylene tip. Once the rats were immobile, the propylene tip was gently pressed against the plantar surface of the hind paw. A single operator performed this procedure to guarantee the same strength of the delivered stimulus. Each hind paw was tested three times, with an interval of approximately 5 minutes. Each single stimulus lasted no longer than 5 seconds, which was sufficient to evoke a visible lifting of the stimulated hind limb after unexpected touch. The corresponding force was recorded (in grams). When a lower force applied was capable of producing a nociceptive response, inducing paw withdrawal, it indicated a reduction in the threshold for mechanical stimuli [24].

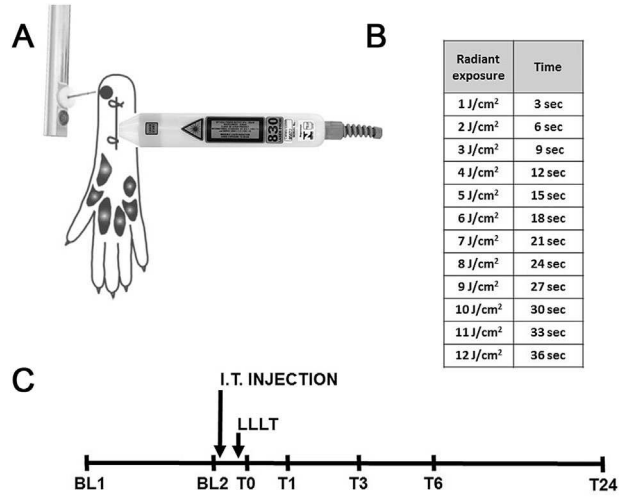
Hot Plate Test

The hot plate was an electrically heated surface kept at a constant temperature of $50.0 \pm 0.5^{\circ}$C. Rats ($n = 8$ per group) were placed on a heated surface within plexiglass walls to constrain their locomotion on the plate. The latency to a discomfort reaction (licking of the paws or jumping) was recorded before and after the surgical procedure, as well as after LLLT. A cut-off time of 20 seconds was chosen to indicate complete analgesia and to avoid tissue injury. The latencies for paw licking or jumping were recorded for each animal [25].

LASER Treatment Procedures

For laser therapy application, the animals were anesthetized with isoflurane (3%). A Low-intensity gallium-aluminium-arsenate (GaAlAs) laser equipment (Ibramed® Equipamentos Médicos, Amparo, Brazil) was used with the following parameters: wavelength of 830 nm (in continuous-mode), radiant exposure of 1–12 J/cm^2, power of 30 mW, irradiation area of 6 mm^2, and duration from 3 to 36 seconds on the hindpaw, as listed in Figure 1A.

To determine the optimum analgesic dose for this model, additional animal groups were used in the first experiment at 1–12 J/cm^2, as listed in Figure 1B. For these groups only the paw mechanical sensitivity was tested 24 hours after LLLT. To investigate whether isoflurane anaesthesia



Radiant exposure	Time
1 J/cm²	3 sec
2 J/cm²	6 sec
3 J/cm²	9 sec
4 J/cm²	12 sec
5 J/cm²	15 sec
6 J/cm²	18 sec
7 J/cm²	21 sec
8 J/cm²	24 sec
9 J/cm²	27 sec
10 J/cm²	30 sec
11 J/cm²	33 sec
12 J/cm²	36 sec

Fig. 1. Laser treatment procedures. (**A**) Application site, (**B**) application time, and dose and experimental protocol (**C**).

would affect LLLT effect, a group of animals ($n = 8$) was treated with the LLLT device turned off (0 J/cm^2), and the animals were restrained for 36 seconds, which is the same amount of time necessary to reach 12 J/cm^2 with the LLLT probe slightly touching the irradiation site.

The experimental protocol used in this study is summarized in Figure 1C. The animals ($n = 8$ per group) were randomly divided into two groups, (i) laser on group, pretreated with intrathecal saline, naloxone or methysergide, and treated with unilateral LLLT; and (ii) laser off group, pretreated with intrathecal saline, naloxone or methysergide and with the laser device turned off while the probe was held in contact. The groups were evaluated before (BL1) and 2 hours after the surgical procedure (BL2) as well as 0, 1, 3, 6, and 24 hours after LLLT.

Cytokine Measurements

Blood samples were collected 24 hours after the incision and centrifuged at 3000 rpm and 4°C for 10 minutes. The TNF-α and IL-1β levels in serum were measured by enzyme-linked immunosorbent assay (ELISA) kits (R&D System, Minneapolis, MN) according to the manufacturer's instructions and guidelines. Briefly, one-hundred microliters of recombinant rat cytokine standard or sample was added to each well of microtiter plates, which had been precoated overnight with sheep anti-rat polyclonal antibody and incubated overnight at 4°C. The following day sheep anti-rat biotinylated polyclonal antibodies were added (1:2000 dilution), and the samples were incubated at room temperature (~22°C) for 1 hour. One-hundred microliters of streptavidin-horseradish peroxidase (1:10000 dilution, Sigma–Aldrich, St. Louis, MO) was then added to each well, at room temperature. After 30 minutes, the plates were washed, and the color reagent α-phenylenediamine dihydrochloride (40 μg in 100 μl per well, Sigma–Aldrich, St. Louis, MO) was added. The reaction was terminated with H_2SO_4 (98 g in 150 μl per well) and the optical density was measured at 490 nm. Each sample was measured in duplicate, and concentrations are expressed as pg.ml^{-1}.

Statistics

Data were analyzed using the Graph Pad software program version 6.0 and expressed as the mean ± S.E.M. Statistically significant differences between the groups were calculated using analysis of variance (ANOVA) followed by the Bonferroni post hoc test. P-values < 0.05 were considered statistically significant.

RESULTS

The Changes Induced by LLLT on the Post Incision Pain

The effects produced by LLLT at 1–12 J/cm^2 for the mechanical threshold 24 hours post-incision are shown in Figure 2. A significant reduction in the threshold of the incised paw was observed 24 hours after the incision compared with the naïve group. No effect was observed after 24 hours. The threshold remained unchanged in the 0 J/cm^2 treated animals. Rats treated with LLLT at 3 or 8 J/cm^2 after 2 hours of surgery had a significant difference compared to 0 J/cm^2 treated rats, and the threshold, measured 24 hours after the incision, was significantly increased compared to the control group. In contrast, rats treated with 1–2, 4–7, or 9–12 J/cm^2 after the incision had a non-significantly different threshold from the control group. The bars in Figure 2 were significantly different for the treatments ($F_{14,89} = 14.71$, $P < 0.01$).

The Changes Induced by Intrathecal Naloxone and Methysergide on the Effect of LLLT at 3 or 8 J/cm^2 on the Mechanical Threshold After Post-Incision Pain

The time course of the effects produced by LLLT at 3 and 8 J/cm^2 on the mechanical threshold is shown in Figure 3. All rats received saline, naloxone hydrochloride (20 μg/5 μl) or methysergide maleate (30 μg/5 μl) intrathecally 15 minutes before the LLLT. The groups also did not differ significantly regarding the BL1 thresholds. Two hours after the incision (BL2), a significant reduction in the threshold of the incised paw was observed in all experimental conditions. The threshold remained unchanged and below BL1 throughout the period of observation in animals treated with 0 J. Rats pretreated with saline and that underwent LLLT at 3 J/cm^2 after the incision had a significantly higher mechanical threshold than the control group (saline and 0 J/cm^2) at all time points after the incision (Fig. 3A). Moreover, animals pretreated with methysergide and that underwent LLLT at 3 J/cm^2 after the incision had a higher mechanical threshold than controls at all time points after the incision. In contrast, naloxone pretreated rats that underwent LLLT at 3 J/cm^2 were not significantly different from the control during the same period. The curves were significantly different for the treatments ($F_{5,30} = 19.31$; $P < 0.01$) and time

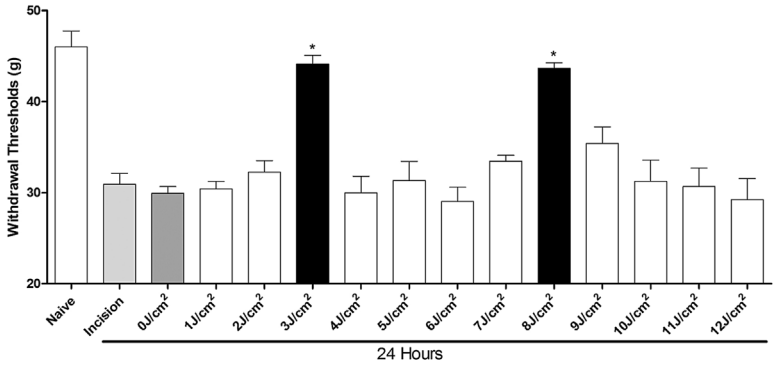


Fig. 2. The effects produced by LLLT at 1–12 J/cm^2 on the mechanical threshold 24 hours after incision. Mechanical withdrawal threshold of naïve animals (non-operated), animals submitted to an incision without LLLT treatment and incised animals that underwent LLLT at 0–12 J/cm^2. Bars indicate the means ± SEM of eight rats per group. $^*P < 0.05$ compared with the incision group.

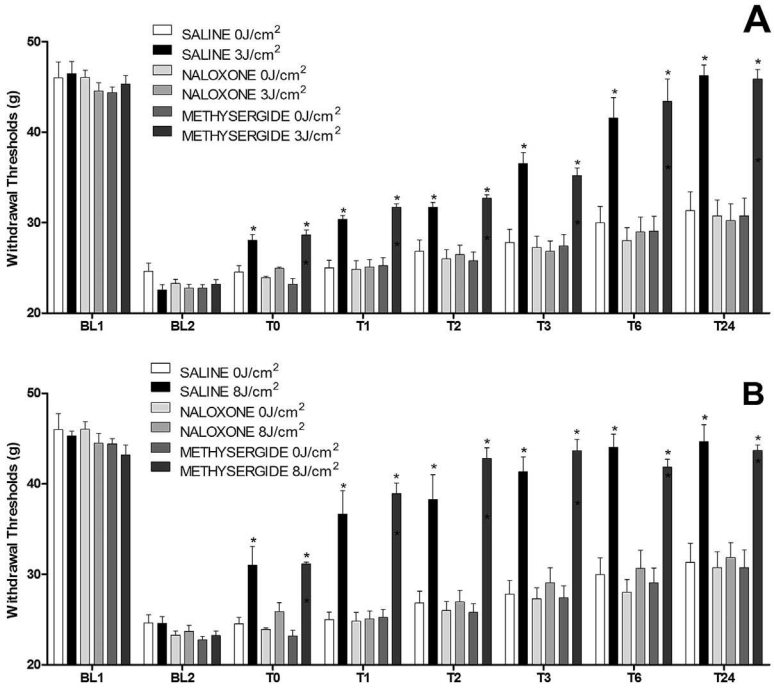


Fig. 3. Effect of LLLT at 3 J/cm^2 (**A**) or 8 J/cm^2 (**B**) on the postoperative mechanical threshold. The experiment was conducted before (BL1) and after plantar incision (BL2). The animals were anesthetized with isoflurane for 20 minutes and pretreated intrathecally with saline, naloxone, or methysergide; 15 minutes later, they were treated with LLLT. The mechanical paw withdrawal latency was measured on incised hind paws 5 minutes after LLLT (T0) and at different times, T1, T2, T3, T6, and T24 hours later. Bars are given as the means ± SEM of eight rats per group. $P < 0.05$ compared with the control group (*).

($F_{7,210} = 333.94$; $P < 0.05$), and they had a treatment x time interaction ($F_{35,210} = 9.52$; $P < 0.05$), as shown in Figure 3A.

Furthermore, rats pretreated with saline that underwent LLLT at 8 J/cm^2 after the incision had a higher mechanical threshold than the control group (saline and 0 J/cm^2) at all time points post the incision (Fig. 3B). Animals pretreated with methysergide that underwent LLLT at 8 J/cm^2 after the incision had a higher mechanical threshold than the control group at all time points post-incision. In contrast, naloxone pretreated rats that underwent LLLT at 8 J/cm^2 had a non-significantly different threshold from the control group during the same period. The curves were significantly different in

terms of the treatments ($F_{5,30} = 42.04$; $P < 0.01$) and time ($F_{7,210} = 173.52$; $P < 0.05$), and there was a treatment x time interaction ($F_{35,210} = 8.29$; $P < 0.05$), as shown in Figure 3B.

The Changes Induced by Intrathecal Naloxone and Methysergide on the Effect of LLLT at 3 or 8 J/cm^2 on the Thermal Threshold After Post-Incision Pain

The time course of the effects produced by LLLT at 3 or 8 J/cm^2 on the thermal threshold is shown in Figure 4. All rats received saline, naloxone hydrochloride (20 μg/5 μl) or methysergide maleate (30 μg/5 μl) intrathecally 15 minutes before LLLT. No significant differences were found in terms of the BL1 thresholds. Two hours after the incision (BL2), a significant reduction in the threshold of the incised paw was observed in all experimental conditions. The threshold remained constant and below BL1 throughout the period of observation in animals treated with 0 J/cm^2. Rats pretreated with saline and that underwent LLLT at 3 J/cm^2 after the incision had a significantly higher thermal threshold than the control group (saline and 0 J/cm^2) at all time points after the incision (Fig. 4A). Additionally, animals pretreated with methysergide that underwent LLLT at 3 J/cm^2 after the incision had a higher thermal threshold than the control group at all evaluated times. In contrast, naloxone blocked the effect of LLLT at 3 J/cm^2 on the thermal threshold in the same period. The curves were significantly different in terms of the treatments ($F_{5,30} = 101.78$; $P < 0.01$) and time ($F_{7,210} = 488.96$; $P < 0.05$), and they had a treatment x time interaction ($F_{35,210} = 5.02$; $P < 0.05$), as shown in Figure 4A.

In addition, the animals pretreated with saline and that underwent LLLT at 8 J/cm^2 after the incision had a higher mechanical threshold than that the control group (saline and 0 J/cm^2) for all times after the incision (Fig. 4B). Animals pretreated with methysergide and that underwent LLLT at 8 J/cm^2 after the incision also had a higher thermal threshold than that obtained in the control group (saline and 0 J/cm^2) at all time points after the incision. In contrast, naloxone blocked the effect of 8 J/cm^2 LLLT on the thermal threshold in the same period. The curves were significantly different in terms of treatments ($F_{5,30} = 149.73$; $P < 0.01$) and time ($F_{7,210} = 438.20$; $P < 0.05$), and they had a treatment x time interaction ($F_{35,210} = 6.19$; $P < 0.05$), as shown in Figure 4B.

The Changes Induced by LLLT at 3 or 8 J/cm^2 on Serum Cytokines After Post-Incision Pain

Cytokine IL-1β and TNF-α production was estimated at 24 hours after plantar incision and is shown in Figure 5A and B, respectively. In the LLLT-control groups (0 J/cm^2), there was a significant increase in the levels of cytokine production after plantar incision (349.75 ± 18.08

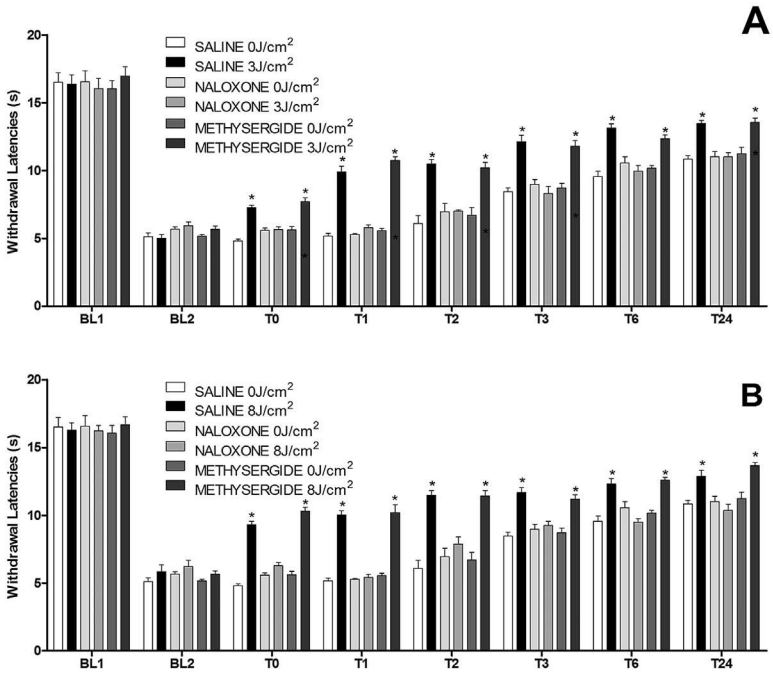


Fig. 4. Effect of LLLT at 3 J/cm^2 (**A**) or 8 J/cm^2 (**B**) on the postoperative thermal threshold. The experiment was conducted before (BL1) and after plantar incision (BL2). The animals were anesthetized with isoflurane for 20 minutes and pretreated intrathecally with saline, naloxone, or methysergide; 15 minutes later, they were treated with LLLT. The thermal paw withdraw latency was measured on incised hind paws 5 minutes after LLLT (T0) and at different times, T1, T2, T3, T6, and T24 hours later. Bars indicate the means ± SEM of eight rats per group. $P < 0.05$ compared with the control group (*).

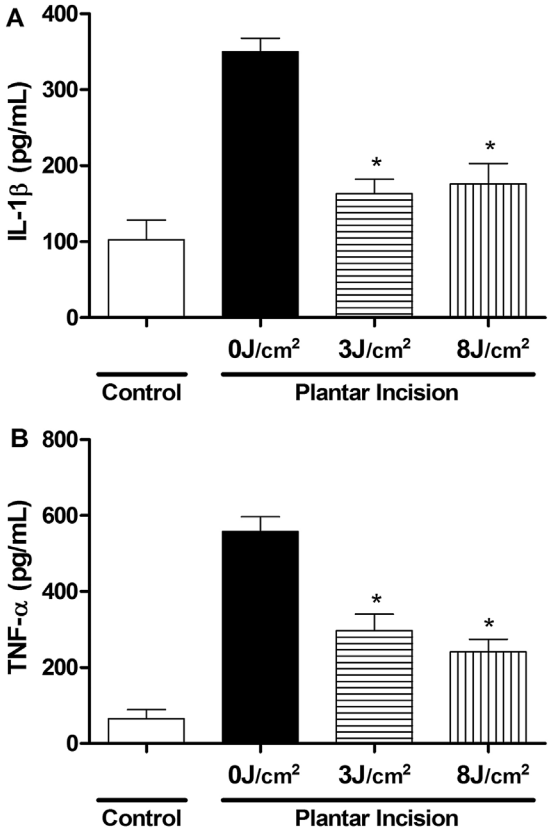


Fig. 5. Effect of LLLT on IL-1β (**A**) and TNF-α (**B**) production 24 hours after plantar incision. Control or incised rats were anesthetized with isoflurane for 20 minutes and treated with LLLT at 0, 3, or 8 J/cm^2. Bars indicate the means ± SEM of eight rats per group. $P < 0.05$ compared with the 0 J/cm^2 group (*).

and 557.87 ± 39.12, respectively, for IL-1β and TNF-α) compared to the control groups (102.44 ± 26.18 and 64.75 ± 24.06, respectively). After LLLT, cytokine production showed a significant decrease in the IL-1β levels (163.07 ± 19.15 and 175.91 ± 26.99 for LLLT at 3 and 8 J/cm^2, respectively) and TNF-α (297.30 ± 40.00 and 240.65 ± 34.31 for LLLT at 3 J/cm^2 and 8 J/cm^2, respectively) compared to the levels of cytokines in the plantar incision group (LLLT at 0 J J/cm^2). The bars in Figure 5 were significantly different for the treatments ($F_{3,15} = 21.42$ and 32.44, $P < 0.01$, for A and B, respectively).

DISCUSSION

The current study demonstrates the effects of LLLT at 3 or 8 J/cm^2 on the mechanical and thermal thresholds and serum cytokines in an incisional model of pain. The analgesic effects of LLLT are dose-dependent, and a response is not observed when a sub- or super-dosage is used. However, effective analgesia is observed when used in adequate doses and it is possible to observe a "Therapeutic Window" for effective photobiostimulation [20,26,27]. In the context of LLLT, this effect possible indicates an hormesis dose-response [28]. This "biphasic" response suggests that if insufficient energy is applied there will be no response because the minimum threshold has not been reached; if more energy is applied, a threshold is crossed, and biostimulation is achieved. However, when too much energy is applied, the effect disappears and is replaced by bioinhibition [28,29].

In addition to these observations, previous studies using both human [13,14,30,31] and experimental pain [19,27] have shown that laser radiation parameters used for pain treatment differ significantly if the energy density is in the range from 0.9 to 105 J/cm^2, if power density ranged from 9.8 to 500 mW and if wavelengths were in the range from 632.8 to 904 nm. In our study, when rats were exposed to a dose of 3 or 8 J/cm^2, the incisional pain, reflected in the nociceptive thermal and mechanical thresholds, was significantly attenuated compared to animals treated with 1–2, 4–7, 9–12 J/cm^2 or control with no laser intervention. It is important to note that the effect was observed from the first period following application, and it lasted for 24 hours post-treatment. The hypothesis to explain this effect is neural inhibition/conduction block, an indicator of the potential of LLLT for pain-relieving effects [11]. Neural inhibition would prevent synaptic transmission from the skin to the brain, which ultimately leads to suppression of central sensitization and is associated with long-term pain reduction [32].

The antinociceptive effects observed at 3 or 8 J/cm^2 were not affected by prior administration of methysergide, whereas the anti-hyperalgesic effect at 3 or 8 J/cm^2 after incision was sensitive to naloxone. According Hagiwara et al. [33], the pain reduction caused by the laser at 830 nm may come from effects related to the endogenous opioids release. In support of our results and previous observations, studies have shown that LLLT increases peripheral opioid release through the migration of immune system cells, with local release of beta-endorphin, which is antagonized by naloxone [33–35]. Together, these results reveal an important role of the endogenous opioid system in the anti-nociception mediated by LLLT.

On the other hand, the role of serotonin in LLLT-mediated analgesia had not been well studied. Methysergide is the least selective of the serotonin antagonists because it binds to both 5-HT$_1$ and 5HT-$_2$ receptors binding sites in the brain and spinal cord [36]. Therefore, the endogenous serotonin system could play a role in nociception because it has different functional tissue-specific receptors [37,38]. Because serotonin transport from the periphery to the central nervous system (CNS) is prevented by the blood–brain barrier, there are two distinct, quantitatively very unbalanced compartments where serotonin can exert various effects on pain signaling mechanisms. Peripherally, serotonin triggers excitation and sensitization of primary nociceptive afferent fibers as well as the nociceptive neurons from which these fibers originate in dorsal root ganglia, contributing to peripheral sensitization and hyperalgesia [39]. In the CNS, low doses of serotonin injected via the intrathecal route are known to exert antinociceptive effects, while larger doses induce a pronociceptive effect, and the underlying mechanisms are still not completely understood [40,41]. These

dual actions of serotonin, pro-nociceptive at the periphery and pro- and/or anti-nociceptive at the spinal level, emphasize the complexity of its implications in the neurobiological mechanisms of nociception and possibly explain why the animals pretreated with methylsergide had a higher thermal threshold. Despite this, our results may extend the observations suggesting that the serotonin system is not involved in the LLLT-mediated analgesia at 3 or 8J/cm^2. Our findings are consistent with a previous study that suggested the analgesia induced by the laser is mediated by peripheral opioid receptors and does not seem to interact with peripheral serotonergic receptors [35].

Another possibility is that the analgesic effect of LLLT can be due to subsequent anti-inflammatory (anti-edematogenic) activity involving hyperalgesic mediators and peripheral opioid receptors [42]. Tissue injury or the presence of foreign material initiates a series of pathophysiological events that trigger the release of pain mediators that in turn control the threshold and activation of nociceptors [43].

The present study demonstrates that LLLT at 3 or 8 J/cm^2 induces a significant reduction in the cytokines known to be released at the time of injury, IL-1β and TNF-α. TNF-α is an endotoxin-induced cytokine that causes necrosis and tumors death, and it is also a pro-inflammatory cytokine that is predominantly released by macrophages [44]. The inflammatory activities of IL-1β are partially derived from the transcriptional induction of cytokines, such as TNF-α and interferons [45].

In animal models of acute inflammation, LLLT reduced the levels of IL-1β and TNF-α although the cellular sources of these cytokines were not identified [46–49]. Additional studies suggest that the LLLT mechanism of action is through inhibiting and/or decreasing the concentration of prostaglandin E_2 (PGE_2), cyclooxygenase 2 (COX-2), and histamine [46,50–53]. Reduction of serum IL-1β and TNF-α at 3 or 8 J/cm^2, as shown in this manuscript, may play a role in the anti-nociceptive action of LLLT by ultimately reducing the overall sensitization of nociceptors [54,55]. These results suggest that the use of LLLT for postoperative pain may reduce inflammation, alleviating peripheral sensitization by reducing the level of inflammatory cytokines and chemokines. This mechanism may be more general and underlie the beneficial effects of LLLT on other inflammatory conditions, especially rheumatoid arthritis, and LLLT has some advantages, such as being non-invasive and non-pharmacologic and having a low rate of side effects.

In conclusion, this study is the first to demonstrate that LLLT at 3 or 8 J/cm^2 reduces both mechanical and thermal thresholds as well as serum cytokines in rat post plantar incisions. Our results suggest that LLLT at 3 or 8 J/cm^2 primarily modulates the endogenous opioid system and is not directly mediated by serotonergic receptors.

ACKNOWLEDGMENTS

We are grateful for the excellent technical support of Luciana Costa Teodoro and Zélia de Fátima Fernandes. We also thank Fundação de Amparo à Pesquisa de Minas Gerais—FAPEMIG (Scientific Initiation Scholarship Program fellowship, FC) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; Master's Level Fellowship, JRP).

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