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ORIGINAL ARTICLE

Infrared low-level diode laser on inflammatory process modulation in mice: pro- and anti-inflammatory cytokines

Thiago Y. Fukuda · Maury M. Tanji · Suélen R. Silva · Maria N. Sato · Hélio Plapler

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Abstract To evaluate the modulation of proinflammatory (interleukin-6, IL-6; tumor necrosis factor-α, TNF-α; and interferon-γ, IFN-γ) and anti-inflammatory cytokines (transforming growth factor-β1, TGF-β1) in the inflammation processes in vivo with low-level laser action, 50 isogenic mice were randomly distributed into three groups: control (no surgical procedure, n=10), sham (surgical procedure with three standard cutaneous incisions, followed by an abdominal muscle incision and suture, n=20), and laser (same procedure followed by laser exposure, n=20). The sham group was divided into three subgroups: sham I (euthanasia and evaluation, 36 h after surgical procedure), sham II (euthanasia and evaluation, 60 h after surgical procedure), and sham III (euthanasia and evaluation, 84 h after surgical procedure). The laser group was also divided in three subgroups: laser I (a single laser session, 12 h after surgery), laser II (two laser sessions, 12 and 36 h after surgery), and laser III (three laser sessions, 12, 36, and 60 h after surgery). All animals in the laser groups received three points per session of continuous infrared laser (wavelength of 780 nm, power of 20 mW, fluency of 10 J/cm^2, exposure time of 20 s per point, and energy of 0.4 J). After euthanasia, spleen mononuclear cells were isolated and cultured for 48 h. Concentrations of IL-6, TNF-α, IFN-γ, and TGF-β1 were obtained by enzyme-linked immunosorbent assay method. There was a significant difference between the IL-6 and TNF-α concentrations in the 60-and 84-h evaluations when the laser and sham groups were compared to the control group (p<0.05), except for laser II in the TNF-α analysis (p>0.05). The IFN-γ concentration analysis showed a significant difference only in sham II when compared to the control group (p<0.05). Thus, there was a modulatory effect of TNF-α and IFN-γ in the laser group, particularly in the 60-h postoperative evaluation. There was no significant difference between the laser, sham, and control groups for TGF-β1 analysis (p>0.05). The low-level laser application decreased the TNF-α and IFN-γ release in vivo of spleen mononuclear cells in mice, especially after two exposure sessions. However, there was no modulation of the IL-6 and TGF-β1 release.

Keywords Cytokines · Inflammation · Healing · Low-level laser therapy

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Introduction

The low-level laser (LLL) application focusing tissue repair and regeneration is a common practice in the treatment of inflammatory disorders as well as in some infectious conditions. The study of biological and therapeutic effects of the laser with different parameters has been the focus of interest among researchers and clinicians, mainly because the LLL achieves these effects without the risk of overheating and damaging to the irradiated tissue [1–3]. The inflammatory process can be considered a type of local defense

against infectious or irritative consequences after certain traumas. The body produces some proteins or cytokines that enable communication between cells and other organs [4, 5].

Several studies can be found in the literature about the effects of laser irradiation on cells associated with this inflammatory response, as lymphocytes [1, 6], fibroblasts [1, 7], macrophages [8], endothelial cells [9], red blood cells [10], besides components of blood plasma as platelets, hemoglobin, immunoglobulins and proteins [1, 11, 12], cell growth factors[13], and cytokines [3, 14].

In particular, the cytokines, including interleukins, play an important role as mediators of inflammatory responses by acting in the activation of specific cells and accelerating or modulating the inflammation processes [15, 16].

Cytokine is a generic term used to designate a large group of molecules (proteins), produced mainly by T cells, macrophages, and some endothelial cells. The cytokines are involved with signal emissions that promote communication between cells during the onset of immune responses and repair. They can be divided in several categories: interleukin, interferons, transforming growth factor, and tumor necrosis factor, among others [5].

Tumor necrosis factor-α (TNF-α) and some interleukins are known as proinflammatory cytokines, playing an important role as mediators of inflammatory and immunologic processes, proteolysis, cell recruitment, and tissue repair. TNF-α has a key position in the cascade of cytokines release, and it also promotes the stimulation of other cytokines such as interleukin-6 (IL-6). IL-6 has been associated with several diseases that are related to inflammatory processes such as rheumatoid arthritis, acute pancreatitis, viral infections, bacterial meningitis, and Alzheimer's disease [17]. Moreover, these inflammatory mediators sensitize primary afferent nociceptors, thus increasing pain sensitivity [18]. Likewise, interferon-γ (IFN-γ) also plays a key role in different immune and inflammatory responses, especially in the acute stages [5]. In contrast, transforming growth factor-β1 (TGF-β1) is considered an anti-inflammatory cytokine and is commonly associated with negative regulation of the activity of monocytes and lymphocytes [14].

Recent studies have shown that in both conditions, in vitro as in vivo, the LLLT can modulate the responses of tissue repair [19–21], as well as the levels of pro- and anti-inflammatory cytokines [3, 13]. Another important characteristic of LLL is its systemic effects, and evidence on this is shown in metabolic changes in the irradiation site, as well as in more distant areas [11, 22, 23].

The LLL in the red light spectrum seems to change levels of IL-6, TNF-α, and IFN-γ in experimental models with induced inflammation [3, 17, 24]. Corroborating these findings, the application of incoherent polarized visible light (λ of 400 to 2,000 nm) can induce structural and functional modifications in the pro- and anti-inflammatory cytokines, decreasing IL-6, TNF-α, and IFN-γ, as well as increasing TGF-β1 [14] However, the results of the LLL effects in the infrared light spectrum are still conflicting. Bouma et al. [9] demonstrated that the infrared laser (IR) seems to promote modulatory effects on the immune system without changing the levels of IL-6 and TNF-α. In contrast, a study conducted by Yamaura et al. [18] showed that the IR laser irradiated in synovial cells in vitro decreases TNF-α, but did not change the levels of IL-6. Partial data from the current study (pilot study) showed that the IR laser (λ of 780 nm) seems to decrease the systemic concentration of proinflammatory cytokines in induced injury in mice, after a single session of laser irradiation [25].

Thus, there is evidence in the literature of LLL systemic effects in the modulation of inflammatory processes. However, many discrepancies were found regarding to the optimal parameters of stimulation. This situation demonstrates a clear need for further studies in order to obtain a more scientific basis concerning the variables inherent to the laser irradiation in the tissue repair process and to establish more precise parameters in relation to immediate or cumulative effects.

Therefore, the purpose of this paper was to analyze the immediate and cumulative role of a low-level infrared laser on the proinflammatory (IL-6, TNF-α, and INF-γ) and anti-inflammatory cytokine release (TGF-β1) from spleen mononuclear cells in mice, in vivo.

Methods

This study was approved by the Ethics Committee of the Federal University of São Paulo, protocol 2038/07. The experiments were performed on 50 BALB/C isogenic male mice weighing 25–30 g each. The animals were maintained in appropriate cages with a 12-h light/dark cycle, with a temperature around 20 °C, with a relative humidity of 65 %, and with access to food and water ad libitum.

Surgical procedure

Anesthesia protocol consisted of a ketamine (0.06 mL) and xylazine 2 % (0.015 mL) application by intraperitoneal injection. The animals were positioned on a flat surface with their limbs in extension position and shaved. A skin square flap measuring 2 cm each side was created, yet the cranial base of the flap was kept intact (Fig. 1a). The abdominal muscle under the flap was exposed and the linea alba was incised in a 1-cm extension; both muscle and skin flap were sutured with a monofilamentar thread (mononylon 6–0) [25].

From the 50 animals studied, 40 were submitted to this surgical procedure, and afterwards, they were randomly assigned to a sham group or to a LLL group. The animals

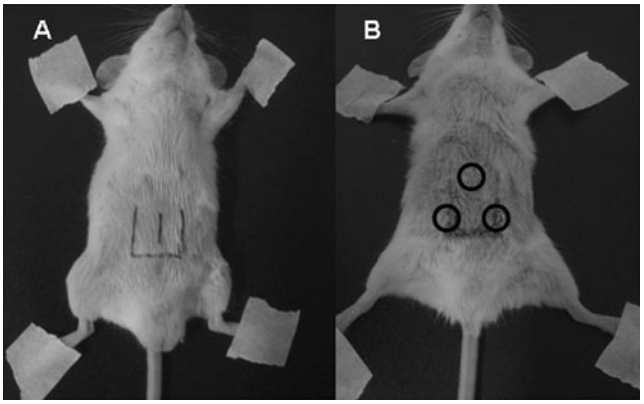

Fig. 1 **a** Planning of the surgery with three standardized cutaneous incision and posterior 1-cm incision in the abdominal muscle. **b** LLL exposure points after surgical proceeding [25]

of the LLL group (n=20) were randomly distributed into three subgroups (Fig. 2):

Laser I: Eight animals with one laser exposure 12 h after surgical procedure

Laser II: Six animals with two laser exposures 12 and 36 h after surgical procedure

Laser III: Six animals with three laser exposures 12, 36, and 60 h after surgical procedure

For each condition, the same surgical procedure and assessment were performed for a sham group (n=20) but without laser application (sham I, II, and III). The ten remaining animals served as a control group, i.e., without surgical procedure in order to obtain baseline levels of cytokines.

LLL irradiation

An infrared AsGaAl diode laser (model Twin Laser) with a λ value of 780 nm, a spot size of 0,04 cm^2, and an output power of 20 mW was used. Three punctual laser applications were performed on the exposed area at the moment 12 h (laser I), 12 and 36 h (laser II), and 12, 36, and 60 h (laser III) after the surgical procedure (Fig. 1b). Only a simulation was performed of the laser application in the animals in the sham groups (I, II, and III), i.e., the laser device was kept in the standby mode. Contact techniques were used in all applications. The LLL exposure to the laser groups was continuous with an energy density of 10 J/cm^2, an application time of 20 s, and a final energy of 0.4 J per point. It is important to highlight that we used a new device which came with a calibration certificate. However, we also evaluated the output power previously to the study with a power meter (LaserCheck, Coherent, USA) and the equipment showed exactly the same output power of 20 mW. The same procedure was also done during the experiments [26]. Laser irradiation was not utilized with the control group.

Separation of mononuclear cells

The animals were euthanized always 24 h after the last LLL exposure in laser groups (I, II, and III); the spleen was dissected and macerated and later mononuclear cells were isolated using Ficoll-Hypaque at a density of 1.095. The euthanasia in the sham groups followed the same period of time as in the designated laser groups. These cells were counted in hematologic automated equipment, and the concentration was adjusted to 2.0×10^6 cells/mL. The cells were incubated in a 96-well culture plate and stimulated with concanavalin mitogen (ConA) for 48 h. After this period, the supernatant was removed and frozen to −80 °C until the dosage of cytokine was produced.

Enzyme-linked immunosorbent assay method analysis

Analyses were carried out in the laser and sham groups using specific antigens of IL-6, TNF-α, IFN-γ, and TGF-β1 cytokines dosage, based on the enzyme-linked immunosorbent assay (ELISA) test (*eBioscience*, *Inc*., San Diego, CA). A control group, i.e., without surgical procedure, was also evaluated to obtain normal values.

The plates were coated with capture antibody and incubated overnight at 48 °C. The wells were blocked with assay diluent. All samples were incubated overnight at 48 °C. The wells were washed out and the detection antibody was added and incubated at room temperature for 1 h. The wells were washed out and avidin–HRP complex was added and incubated at room temperature for 30 min. Afterwards, the substrate solution was added and incubated at room temperature for 15 min. The stop solution was added and the plate was read at 450 nm.

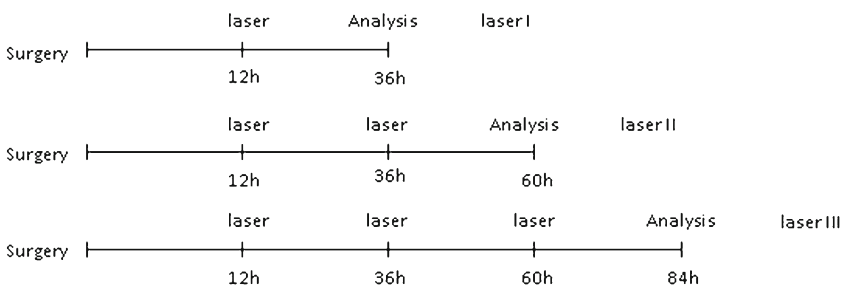

Fig. 2 Study design for the laser groups

Analyses of data

In the beginning, a normality and homogeneity of variance test (Anderson–Darling and Levene's test) was performed followed by a Mann–Whitney test designed to compare the experimental groups (sham and laser) versus the control group. Analysis of variance utilizing the Kruskal–Wallis multiple comparison tests was used to compare these experimental groups at three different evaluation times (36, 60, and 84 h). Finally, the Mann–Whitney test was again used for comparison between laser and sham groups at each time period. The data demonstrate a mean, median, standard deviation (SD), standard error of mean (SEM), confidence interval of 95 %, and a statistical significance that was considerate at $p<0.05$. The Statistical Package for Social Sciences version 16.0 was used for data processing.

Results

IL-6 concentration

Table 1 shows the comparison of the IL-6 concentration in picograms per milliliter between the experimental groups (sham and laser) and the control group. The sham II, laser II, sham III, and laser III groups showed a higher IL-6 concentration when compared to the control group ($p<0.05$). No significant differences were found in the comparison between the sham I and laser I groups versus the control group ($p>0.05$).

In the analysis between the three different times of euthanasia (36, 60, and 84 h) for the experimental groups, there was no significant difference for the sham ($p=0.069$) and laser groups ($p=0.480$). In the comparison between laser and sham groups for each specific time period (36, 60, and 84 h), no significant differences were found between sham I and laser I ($p=0.654$) and between sham III and laser III groups ($p=0.589$). The comparison between sham II and laser II groups showed a greater IL-6 concentration in the sham II group ($p=0.065$), but this difference was not statistically significant (Fig. 3).

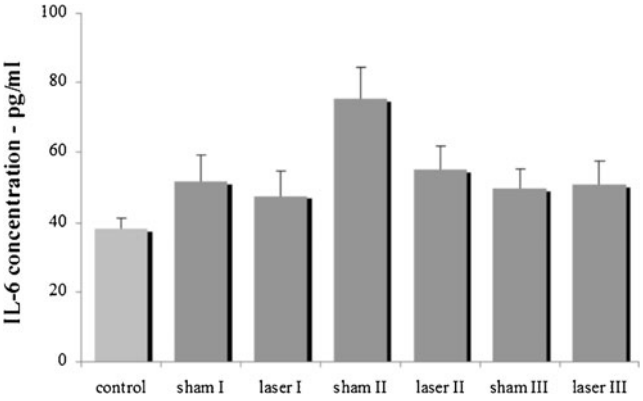

Fig. 3 Average (±SEM) of the IL-6 concentration in picograms per milliliter released by the mononuclear spleen cells of the control, sham, and laser groups. There was no significant difference ($p>0.05$, Kruskal–Wallis test) between the 36, 60, and 84 h of evaluation; there was no significant difference ($p>0.05$, Mann–Whitney test) in the comparison between sham I versus laser I, sham II versus laser II, and sham III versus laser III

TNF-α concentration

Table 2 shows the comparison of the TNF-α concentration in picograms per milliliter between the experimental groups (sham and laser) and the control group. The sham II, sham III, and laser III groups showed a significantly higher TNF-α concentration when compared to the control group ($p<0.05$). No significant differences were found in the comparison between the sham I, laser I, and laser II groups versus the control group ($p>0.05$).

In the analysis between the three different times of euthanasia (36, 60, and 84 h) for the experimental groups, there was a significant difference for the sham ($p=0.030$), because the sham III group showed a higher TNF-α concentration when compared to the sham I group ($p=0.008$). Moreover, we also found a significant difference for the laser ($p=0.012$), because the laser III group showed a higher TNF-α concentration in relation to the laser I group ($p=0.001$). In the comparison between laser and sham groups for each specific time period (36, 60, and 84 h), no significant differences were found between sham I and laser I groups ($p=$

Table 1 Results of IL-6 concentrations in picograms per milliliter released by the mononuclear spleen cells of the control, sham, and laser groups

IL-6	Control	Sham I	Laser I	Sham II	Laser II	Sham III	Laser III
Mean	38.0	51.6	47.6	75.4	55.3	49.8	50.9
Median	38.8	48.4	44.4	85.4	58.0	50.5	49.7
SD	9.9	21.7	19.8	21.5	15.9	13.4	16.2
SEM	3.1	7.7	7.0	8.8	6.5	5.5	6.6
CI (95 %)	6.1	15.0	13.7	17.2	12.7	10.7	13.0
p value[a]	X	0.130	0.302	0.005*	0.030*	0.031*	0.039*

*$p<0.05$ (significant difference when compared to control)

[a]Comparison of each experimental group versus control (Mann–Whitney test)

Table 2 Results of TNF-α concentration in picograms per milliliter released by the mononuclear spleen cells of the control, sham, and laser groups

TNF-α	Control	Sham I	Laser I	Sham II	Laser II	Sham III	Laser III
Mean	51.1	58.9	51.3	97.4	78.5	99.0	103.0
Median	56.5	58.3	49.0	100.8	94.0	112.1	103.9
SD	15.0	16.8	10.8	37.2	37.2	22.0	12.2
SEM	4.7	6.0	3.8	15.2	14.1	9.0	5.0
CI (95 %)	9.2	11.6	7.5	29.8	27.5	17.6	9.8
p value[a]	X	0.320	0.789	0.017*	0.110	0.002*	0.001*

*$p<0.05$ (significant difference when compared to control)

[a]Comparison of each experimental group versus control (Mann–Whitney test)

0.328), sham II and laser II groups ($p=0.628$), and sham III and laser III groups ($p=0.937$) (Fig. 4).

IFN-γ concentration

Table 3 shows the comparison of the IFN-γ concentration in picograms per milliliter between the experimental groups (sham and laser) and the control group. Compared to the control group, only the sham II group showed a significantly higher IFN-γ level ($p<0.05$). No significant differences were found in the comparison between the sham I, laser I, laser II, sham III, and laser III groups versus the control group ($p>0.05$).

In the analysis between the three different times of euthanasia (36, 60, and 84 h) for the experimental groups, there was a significant difference for the sham group ($p=0.046$), because the sham II group showed higher IFN-γ concentration when compared to the sham I ($p=0.030$) and sham III groups ($p=0.041$). There was no significant difference for the laser I, II, and II groups ($p=0.082$). In the comparison between sham and laser groups for each time period (36, 60, and 84 h), no significant differences were found between sham I and laser I groups ($p=0.195$) and between the sham III and laser III groups ($p=0.588$). In the comparison between the sham II and laser II groups, a higher IFN-γ concentration was found in the sham II group ($p=0.065$), but this difference was not statistically significant (Fig. 5).

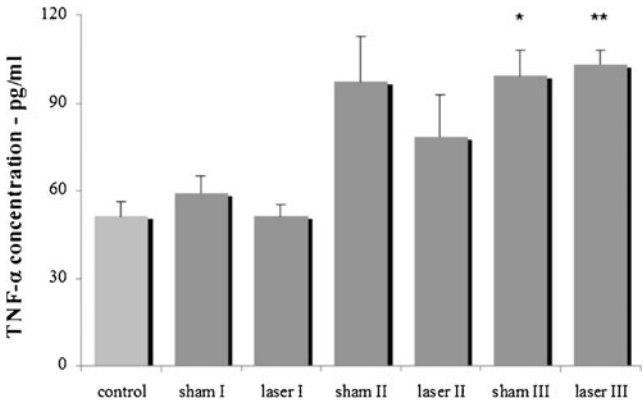

Fig. 4 Average (±SEM) of the TNF-α concentration in picograms per milliliter released by the mononuclear spleen cells of the control, sham, and laser groups. There was no significant difference ($p>0.05$, Mann–Whitney test) in the comparison between sham I versus laser I, sham II versus laser II, and sham III versus laser III groups; * there was significant difference between ($p=0.008$, Kruskal–Wallis test) between sham I (36 h) and sham III (84 h); ** there was significant difference ($p=0.001$, Kruskal–Wallis test) between laser I (36 h) and laser III (84 h)

TGF-β1 concentration

Table 4 shows the comparison of the TGF-β1 concentration in picograms per milliliter between the experimental groups (sham and laser) and the control group, and there was no significant difference for any of the conditions ($p>0.05$).

In the analysis between the three different times of euthanasia (36, 60, and 84 h), there was no significant difference for the sham ($p=0.157$) and laser groups ($p=0.370$). In the comparison between sham and laser groups for each time period (36, 60, and 84 h), no significant differences were found between the sham I and laser I groups ($p=0.128$), sham II and laser II groups ($p=0.366$), and between the sham III and laser III groups ($p=0.937$) (Fig. 6).

Discussion

The photochemical, photophysical, and photobiological effects generated by the LLL can affect not only the application area but also the surrounding region [27]. The effects of metabolic mediators can reach the most distant areas of the body, generating "systemic effects" [22, 23, 27, 28]; however, few studies prove this hypothesis. A possible explanation is the fact that the tissue submitted to laser irradiation produces signaling factors that, after application, will circulate into the lymphatic system and blood vessels [29, 30].

Thus, the exploration of the modulator mechanism of laser irradiation on communicating or signaling proteins in the repair of inflammatory processes is necessary for

Table 3 Results of IFN-γ concentration in picograms per milliliter released by the mononuclear spleen cells of the control, sham, and laser groups

IFN-γ	Control	Sham I	Laser I	Sham II	Laser II	Sham III	Laser III
Mean	473.6	577.7	470.4	1,331.3	757.6	626.1	721.7
Median	410.6	437.1	394.7	1,308.5	602.9	521.3	745.1
SD	261.0	232.9	136.3	587.4	356.5	294.3	253.7
SEM	82.5	102.7	48.2	239.8	145.5	120.1	103.6
CI (95 %)	159.9	161.3	94.4	469.9	285.2	235.4	203.0
p value[a]	X	0.360	0.574	0.007*	0.128	0.232	0.073

*$p<0.05$ (significant difference when compared to control)

[a]Comparison of each experimental group versus control (Mann–Whitney test)

the better understanding of these systemic effects, providing new opportunities for treatment with LLL. In the present study, the infrared LLL reduced the concentration of TNF-α and IFN-γ proinflammatory cytokines released by mononuclear spleen cells in mice after standardized surgical procedure, especially after the two irradiation sessions.

The ELISA assay was employed for analysis of the pro- and anti-inflammatory cytokine levels, according to Aimbire et al. and Yamaura et al. [18, 31]. This is a standardized and reliable method to measure the cytokine concentration, and for this reason, it was employed in the present study [25, 32, 33]. The results demonstrated that the surgical procedure increased the cytokine release, since the sham II and III groups presented significantly increased concentration of IL-6 and TNF-α as compared to the control groups at 60 and 84 h after surgery. In relation to the IFN-γ concentration, the sham II group also showed a higher concentration when compared to the control or baseline. The lack of changes in the TNF-α and IFN-γ levels in the laser II group when compared to the control group shows that LLL can reduce the proinflammatory cytokine release.

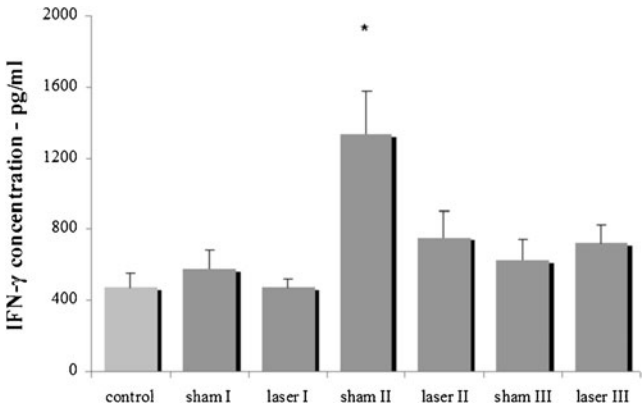

Fig. 5 Average (±SEM) of the IFN-γ concentration in picograms per milliliter released by the mononuclear spleen cells of the control, sham, and laser groups. There was no significant difference ($p>0.05$, Mann–Whitney test) in the comparison between sham I versus laser I, sham II versus laser II, and sham III versus laser III; * there was significant difference ($p=0.030$ and $p=0.041$, respectively, Kruskal–Wallis test) between sham II (60 h) versus sham I (36 h) and sham III (84 h)

Thus, we looked for evidence of the immediate or cumulative laser effects after single or multiple sessions of treatment while attempting to observe differences in the cytokine modulation. Studies have shown a decrease of proinflammatory cytokines immediately after laser irradiation and increased anti-inflammatory cytokines during consecutive applications [14, 31, 34]. However, other studies have shown systemic effects of LLL, but without changing the cytokine levels [35, 36]. The results of our pilot study showed decreased IL-6 and TNF-α concentration with a single LLL session [25]. However, with an increased number of animals, this tendency was not confirmed. Thus, we did not find significant differences in both pro- and anti-inflammatory cytokine release at the evaluation time period of 36 h after surgery. In contrast, this study showed a decrease of proinflammatory cytokines after two sessions, i.e., at the evaluation time period of 60 h after the surgical procedure.

According to the literature, the peak of inflammatory process may occur around 4–6 h after injury [37, 38], and the proinflammatory cytokines seem to achieve the higher concentration around 24 h [16, 39]. In the studies conducted by Aimbire et al. [31] and Albertini et al. [40], the laser was employed immediately after carrageenan-induced inflammation and, therefore, the animals did not suffer traumatic injury, nor vascular damage. The experimental model of the present study was applied in an attempt to simulate surgical proceedings with strong vascular damages in humans. We hypothesized that the LLL application in the peak of inflammatory process could increase the hemorrhage, and for this reason, the first laser irradiation was performed 12 h after the surgical procedure.

The decrease of the TNF-α release can be promoted by several kinds of treatment. Among these therapies, the LLL has characteristics favorable to its use, as a simple application and a noninvasive method to inflammatory modulation [30, 40, 41].

IFN-γ plays an important role in different immune and inflammatory responses, as well as in regulating

Table 4 Results of TGF-β1 concentration in picograms per milliliter released by the mononuclear spleen cells of the control, sham, and laser groups

TGF-β1	Control	Sham I	Laser I	Sham II	Laser II	Sham III	Laser III
Mean	2,879.1	2,259.4	3,170.2	3,612.1	2,687.6	2,646.9	2,419.3
Median	3,075.1	1,803.2	3,078.4	3,879.8	1,598.8	2,977.8	2,206.9
SD	1,149.5	1,427.8	1,273.5	1,059.3	1,642.5	940.1	1,637.2
SEM	363.5	539.6	481.3	432.5	620.8	383.8	668.4
CI (95 %)	704.1	988.9	882.0	847.4	1,214.8	752.1	1,309.8
p value[a]	X	0.363	0.494	0.263	0.796	0.678	0.634

[a]Comparison of each experimental group versus control (Mann–Whitney test)

chemokine secretion [27]. Another proinflammatory cytokine, the IL-6, is a pleiotropic cytokine with a wide range of biological activities. Therefore, this helps the erythropoietic function and controls the immune system response and the production of acute phase reactions, such as the stimulation of C-reactive protein release. Due to these important roles in the early stages of the repair process, these proinflammatory cytokines were analyzed.

There is no standardization of parameters with respect to the laser used in the modulation of inflammatory processes, especially with the infrared laser, which is widely used in clinical practice due to its greater tissue penetration [42]. However, the studies that specifically employed the infrared laser show contradictory results in the TNF-α and IL-6 modulation, with energy ranging from 0.3 to 0.9 J and fluency from 0.3 to 25 J/cm^2 [3, 9, 19, 35, 36]. In the present study, we used the continuous infrared laser (780 nm) with an average power of 20 mW and fluency of 10 J/cm2. Based on these parameters, an application time of 20 s was used, generating a final energy of 0.4 J per point, and the total exposure time was 60 s [25]. Thus, the rationale for choosing these parameters was based on a comprehensive literature review, and most significant results seem to occur with a wavelength in the near infrared and especially with energy to achieve the therapeutic window (approximately 0.4 J), whereas doses greater than 1 J could be inhibitory for small-sized animals such as mice [3, 9, 19, 25, 35, 36].

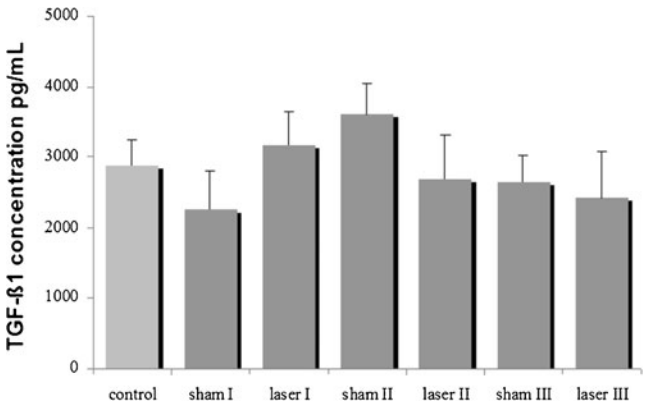

Fig. 6 Average (±SEM) of the TGF-β1 concentration in picograms per milliliter released by the mononuclear spleen cells of the control, sham, and laser groups. There was no significant difference ($p>0.05$, Mann–Whitney test) in the comparison between sham I versus laser I, sham II versus laser II, and sham III versus laser III; there was no significant difference ($p>0.05$, Kruskal–Wallis test) between the 36, 60, and 84 h of evaluation

We did not find an immediate modulation of IL-6, TNF-α, and IFN-γ with a single session of infrared LLL when compared to the sham group in this experimental model. Some factors may have influenced these results, such as an insufficient abdominal muscle injury needed to generate a vigorous inflammatory process and consequently a strong increase in the cytokine release from the mononuclear spleen cells. Perhaps a more aggressive injury with stronger systemic inflammatory stimulus induced more evident changes that could respond positively to a single LLL session. However, analyzing the responses of the laser group, especially after two LLL sessions, we can observe an inhibitory effect for TNF-α and IFN-γ, as well as a tendency for IL-6.

Finally, the modulatory response of the laser in the TGF-β1 anti-inflammatory cytokine was also evaluated at 36, 60, and 84 h after surgery. Unlike previously expected, the LLL did not increase the concentration of this cytokine with a single session (immediate effect), nor with two or three sessions (cumulative effect). One hypothesis for these findings is that the previous modulation of the proinflammatory cytokines by the laser may have inhibited the consequent overproduction of TGF-β1.

Conclusion

The cytokine analysis showed a cumulative effect of the infrared laser after two sessions mainly by decreasing the TNF-α and IFN-γ release of the mononuclear spleen cells in mice. There was no modulation of the IL-6 concentration (despite a tendency) and TGF-β1.

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