Anti-Inflammatory Effects of Low-Level Laser Therapy (660 nm) in the Early Phase in Carrageenan-Induced Pleurisy in Rat

Emerson S. Boschi, MS, Carlos E. Leite, MS, Vasyl C. Saciura, MS, Eduardo Caberlon, MS, Adroaldo Lunardelli, MS, Shanna Bitencourt, MS, Denizar A.S. Melo, PhD, and Jarbas R. Oliveira, PhD*
Laboratório de Pesquisa em Biofísica Celular e Inflamação, Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Av. Ipiranga 6681 prédio 12C sala 263, CEP 90619-900 Porto Alegre, RS, Brazil

Background and Objective: In the classic model of pleurisy there is little evidence about the anti-inflammatory effects of low-level laser therapy (LLLT) as well the dosage characteristics, such as wavelength, total energy, number and pattern of treatment. In this study we investigated the potential effects of LLLT on modulating the pro-inflammatory and anti-inflammatory mediators of acute inflammation in a rat pleurisy model.
Study Design/Materials and Methods: A sample of 48 female Wistar rats were divided into control and experiential groups. An inflammation was induced by carrageenan (0.2 ml) injected into the pleural cavity. At 1, 2, and 3 hours after induction a continuous wave (20 mW) diode laser of the InGaAlP (660 nm) type was used in the four laser groups with different doses and treatment patterns. One group received a single dose of 2.1 J and the other three groups received a total energy of 0.9, 2.1, and 4.2 J. Four hours later the exudate volume, total and differential leukocytes, protein concentration, NO, IL-6, IL-10, TNF-α, and MCP-1 were measured from the aspirated liquid.
Results: All the treatment patterns and quantity of energy studied show significant reduction of the exudate volume ($P<0.05$). Using energy of 0.9 J only NO, IL-6, MCP-1 and IL-10 are significantly reduced ($P<0.05$). On the other hand, higher energies (2.1 and 4.2 J) significantly reduce all variables independently of the treatment pattern. The neutrophil migration has a straight correlation with the TNF-α ($r=0.551$) and NO ($r=0.549$) concentration.
Conclusions: LLLT—660 nm induced an anti-inflammatory effect characterized by inhibition of either total or differential leukocyte influx, exudation, total protein, NO, IL-6, MCP-1, IL-10, and TNF-α, in a dose-dependent manner. Under these conditions, laser treatment with 2.1 J was more effective than 0.9 and 4.2 J. Lasers Surg. Med. 40:500–508, 2008. © 2008 Wiley-Liss, Inc.

Key words: inflammation; LLLT; pleurisy

INTRODUCTION

Inflammation is a protective process essential for the preservation of the integrity of the organisms in the event of physical, chemical and infectious damage [1]. Acute inflammation is characterized by the classical signs of pain, heat, redness and swelling, involving a complex series of events including vasodilatation, increased permeability, fluid exudation and migration of leukocytes to the site of the inflammation [2].

Carrageenan-induced pleurisy is a well-characterized experimental model of inflammation that permits the quantification and correlation of both exudate and cellular migration with changes of other inflammatory parameters [3]. The major characteristic of this model in the rat is the biphasic profile of the inflammatory reaction, where early (4 hours) and late (48 hours) phases of both cell migration and exudation are clearly observed [4]. Thus, this model constitutes a biologic system suitable for the investigation of possible correlation occurring between cell migration, fluid leakage, nitric oxide (NO), chemokine, pro-inflammatory and anti-inflammatory cytokines.

One of the early cellular events in inflammation is the migration of leukocytes, primarily neutrophils. In addition, NO plays an important role in inflammation such as plasma exudation and leukocyte infiltration. The NO synthesis (NOS) inhibitors can reverse several classic inflammatory symptoms [5].

The maintenance of leukocyte recruitment during inflammation requires intercellular communication between infiltrating leukocytes and the endothelium. These events are mediated by the generation of early response cytokines, for example, interleukin (IL-1) and tumor necrosis factor (TNF-α), the expression of cell–surface adhesion molecules and the production of chemotactic molecules, such as chemokines [6].

Chemokines are a family of structurally related glycoproteins with potent leukocyte activation and/or chemotactic activity. Most chemokines are produced in response to a variety of inflammatory stimuli, including the

*Correspondence to: Jarbas R. Oliveira, PhD, Laboratório de Pesquisa em Biofísica e Inflamação, Pontifícia Universidade Católica do Rio Grande do Sul, Av. Ipiranga 6681 prédio 12C sala 263, CEP 90619-900 Porto Alegre, RS, Brazil.
E-mail: jarbas@pucrs.br
Accepted 27 May 2008
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.20658

early-response cytokines, TNF-α, IL-1, C5a, leukotriene B4 and interferons [7]. Monocyte chemoattractant protein-1 (MCP-1) is a chemokine that has been demonstrated to attract monocytes and neutrophils both in vitro and in vivo and appears to be a key part of leukocyte emigration, serving as a promoter of the transition from leukocyte rolling to adhesion on the endothelial surface [8].

Representative inflammatory cytokines include TNF-α, IL-1, IL-6, and IL-8. The TNF-α and IL-1 has been revealed to have many overlapping biological activities in the inflammatory reaction: both cause fever, accumulation of neutrophils in local tissues, induction of vascular adhesion molecules and stimulation of acute phase protein synthesis [9]. Some of these responses by TNF-α and IL-1 are now known to be mediated by other inflammatory mediators or secondarily induced cytokines such as IL-6 [10].

The IL-6 is widely produced by several cells such as fibroblasts, endothelial cells, keratinocytes, monocytes, T cells, mast and tumor cell lines, and cells of neural origin. Thus, IL-6 is often used as a marker for systemic activation of pro-inflammatory cytokines [11]. However, evidence also indicates that, under certain conditions, IL-6 has both pro-inflammatory and anti-inflammatory properties [12]. Apart from this, there is also experimental evidence indicating that IL-6 down-regulates the synthesis of IL-1 and TNF-α [13].

The IL-10 is known as the most important anti-inflammatory cytokine found within the human immune response. Previous data has shown that IL-10 is capable of blocking the inflammatory response induced by several inflammatory stimuli in different models [11].

The conventionally used therapies for inflammation, non-steroidal anti-inflammatory drugs (NSAIDs), have a very important role in managing pain and acute inflammatory conditions [14], though with rather discouraging profile of side effects [13]. Even the anti-inflammatory drugs, cyclo-oxygenase 2 (COX-2) inhibitors, are not devoid of adverse effects [15]. Therefore, alternative physical techniques such as low-level laser therapy (LLLT) have been used clinically, among other indications for its proposed anti-inflammatory effects, pain relief and acceleration of the regeneration of damaged tissues [16]. Although in the past decade several studies have examined the effects of LLLT, the treatment protocols used included enormous variations in parameters (such as wavelengths, energy and power densities, wave modes, number of treatments), which makes it difficult to assess the optimum treatment parameters in each case [17].

However, more recent studies have been able to find some dose-dependent effects on TNF-α [18] and prostaglandin E_2 levels [19], besides reduction of the oedema [20,21], neutrophils migration [21], nitric oxide synthase (iNOS) expression [22] and COX-2 mRNA expression [23] in the different inflammatory experimental models after LLLT. The efficacy of LLLT radiation as an anti-inflammatory therapy is controversial.

Therefore, the present study was designed to explore the potential effects of LLLT applied at different points with dose variation on the modulation of the pro-inflammatory and anti-inflammatory mediators of acute inflammation in carrageenan-induced pleurisy in rat model.

MATERIALS AND METHODS

Animals

Adult female Wistar rats (*Rattus norvegicus*) bred in our laboratory (around 3–4 months old, weighing 180–220 g) were used, all of the same ancestry and socialization with free access to food and water. Animals were divided into six groups composed of eight rats each. The rats were maintained in accordance with the "*Guiding Principles in the Care and Use of Animals*" and the present study was approved by ethics committee of Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS).

Carrageenan-Induced Pleurisy

Rats were anesthetized with a mix of Ketamine (80 mg/kg) and Xilazine (12 mg/kg). Saline 0.2 ml (saline group) or saline containing 2% λ-carrageenan (Cg) 0.2 ml (carrageenan group and Cg+laser groups) was injected into the pleural cavity at the level of the sixth left intercostals space. At 4 hours after the intrathoracic injection, the animals were sacrificed with decapitation and the pleural cavity was opened. The liquid that had accumulated in the pleural cavity was washed with 2.0 ml of sterile saline solution (NaCl 0.9%) containing 1% EDTA in the aspirated liquid. All exudate contaminated with red blood cells was discarded [2].

Laser Irradiation

The continuous wave diode laser of InGaAlP type (model Endophoton-LLT-010-KLD Biosistemas Equipamentos Eletrônicos Ltd., Sao Paulo, Brazil) with an output power of 20 mW and a wavelength of 660 nm (visible red) was used. The spot size was 0.035 cm^2 and the power density was 0.571 W/cm^2. Table 1 shows the laser parameters used in our experiment. Different doses were used for each of the four groups: the group with 1 point (Cg+1 point laser with 21 J/cm^2), the group with 3 points (Cg+3 points laser with 3 J/cm^2 each), the group with 7 points (Cg+7 points laser with 3 J/cm^2 each) and the group with 14 points (Cg+14 points laser with 3 J/cm^2 each). All the experimental groups were irradiated with LLLT from a spot in contact with the skin at 1, 2, and 3 hours after pleurisy induction. Thus, the total energy delivered from the three treatment sessions was 2.1, 0.9, 2.1, and 4.2 J during 37, 16, 37, and 74 seconds, respectively. The animals received the irradiation at the left thoracic wall with different treatment methods according to the group: the group of 1 point was irradiated with LLLT where the pleurisy was induced; the group of 3 points received LLLT around the induction area; the group of 7 points was irradiated, in which 1 point was on the induction local and 6 points were symmetrically distributed around the area; in the group of 14 points they were distributed in a craniocaudal direction of crescent 2, 3, 4, and 5 points involving all thoracic wall.

TABLE 1. Protocol of Laser Radiation

Model	Laser 660 nm + Carrageenan (4 hours)			
Output power (mW)	20 (continuous)			
Spot size (cm^2)	0.035			
Power density (W/cm^2)	0.571			
Groups	1 Point	3 Points	7 Points	14 Points
Energy density per point (J/cm^2)	21	3	3	3
Time per point (seconds)	36.75	5.25	5.25	5.25
Dose per treatment (J/cm^2)	21	9	21	42
Total dose from all three treatments (J/cm^2)	63	27	63	126
Total energy per point (J)	0.7	0.1	0.1	0.1
Total energy per treatment (J)	0.7	0.3	0.7	1.4
Total final energy (J)	2.1	0.9	2.1	4.2

Exudate Analysis

The volume of the exudates was measured and the result expressed by subtracting the volume injected into the pleural cavity (2.0 ml of solution) from the total volume aspirated. Total leukocytes were diluted in Thoma solution (1:20) and counted in a Neubauer chamber using light microscopy. Cytological slide smears stained with May-Grünwald/Giemsa were used for differential leukocyte counts in a light microscope [24]. The pleural liquid removed from the rats was centrifuged at 1,200*g* for 10 minutes and the protein concentration measured by the Biuret technique. NO is a very unstable radical, rapidly metabolized from nitrate to nitrite in the presence of oxygen [25]. Therefore, the amount of NO in the exudate was analyzed using the Griess reaction that measured nitrite, as previously described [26]. The frozen cell-free supernatant of the pleural fluid was thawed, and IL-6, IL-10, TNF-α and MCP-1 were determined using a rat enzyme-linked immunosorbent assay (ELISA) kit (Biosource, Camarillo, CA).

Statistical Analysis

The results were evaluated statistically by analysis of variance (ANOVA) with LSD test post hoc using Statistical Package for the Social Sciences (SPSS) 12.0 software and were expressed as the means $\pm$ standard error of means (SEM). The correlation between the variables and the neutrophil infiltration into the pleural cavity was analyzed by using Pearson Correlation Linear. The level of statistical significance was defined as $P<0.05$.

RESULTS

According to the analysis of this study, the rats injected with carrageenan in the pleural space developed an acute pleurisy after 4 hours with a significant increase in all variables when compared to the saline group.

Figure 1 shows the protective effect of LLLT on the inflammatory parameters, such as: protein concentration, exudate volume, the amount of leukocytes and neutrophils in the pleural liquid of the saline group, carrageenan and in the four groups irradiated with different laser doses and treatment methods.

Protein

Protein concentration (Fig. 1A) showed a significant decrease ($P<0.05$) in laser groups with 1 point (1.01 ± 0.11 g/dl), 7 points (0.94 ± 0.03 g/dl) and 14 points (1.13 ± 0.10) when compared to the carrageenan group (1.42 ± 0.04 g/dl). It was observed that the laser group with 3 points (1.47 ± 0.07 g/dl) did not modify the concentration of proteins that had migrated to the pleural cavity.

Exudate Volume

In relation to the exudate volume (Fig. 1B) collected 4 hours after the pleurisy induction, a significant reduction was observed in the four groups irradiated with LLLT when compared to the carrageenan group (1 point: 0.32 ± 0.03 ml; 3 points: 0.68 ± 0.07 ml; 7 points: 0.60 ± 0.08 ml and 14 points: 0.52 ± 0.06 ml vs. Cg: 0.98 ± 0.04 ml, $P<0.001$). Besides that, the laser group with 1 point (2.1 J) was more effective than the laser group with 7 points (2.1 J) in reducing pleural exudate volume ($P = 0.002$).

Total Leukocytes

All groups irradiated with LLLT, except the group with 3 points, showed a significant decrease in the total number of leukocytes when compared to the carrageenan group (1 point: 35.03 ± 4.07; 3 points: 63.25 ± 5.69; 7 points: 37.21 ± 2.35 and 14 points: 46.22 ± 3.81 vs. Cg: 70.67 ± 3.90, $P = 0.001$) (Fig. 1C). Based on this, the group that received a final dose of 0.9 J (group 3 points) did not demonstrate reduction in the leukocyte cells infiltration to the inflammatory site of the lesion ($P = 0.176$).

Neutrophils

The reduction in the inflammatory acute phase response, represented by a lower migration of polymorphonuclear-neutrophil cells (PMNs), showed a mean of 50% reduction of these cells in the groups 1, 7, and 14 points that received

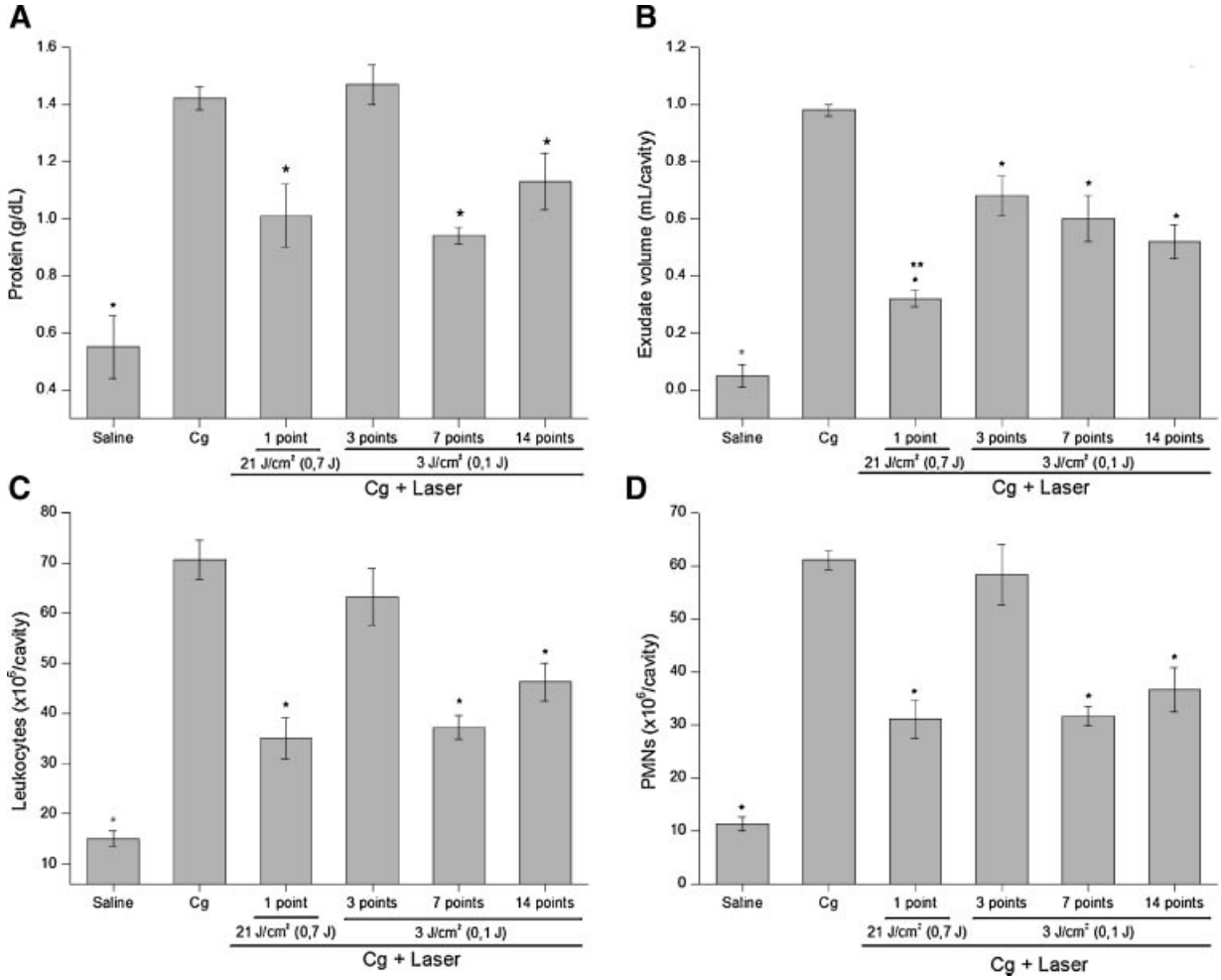

Fig. 1. Effect of low-power laser on the total proteins concentration (**A**), exudate volume (**B**), total leukocytes (**C**) and polymorphonuclears (PMNs) (**D**) that accumulated in pleural cavity after 4 hours of carrageenan injection on the saline, carrageenan (Cg) and laser groups. The Cg+laser groups were irradiated at 1, 2, and 3 hours after induction of the pleurisy with 3 or 21 J/cm^2. The groups with 3 J/cm^2 received the irradiation at 3 points (9 J/cm^2), 7 points (21 J/cm^2), 14 points (42 J/cm^2) per session and the group with 21 J/cm^2 received it at 1 point per session. Results are expressed as means ± SEM of eight animals. *$P<0.05$ versus carrageenan group. **$P<0.05$ 1 point versus 7 points.

higher or equal to 2.1 J energy when compared to the carrageenan group or the laser group with 3 points (1 point: 31.09 ± 3.60; 7 points: 31.61 ± 1.90 and 14 points: 36.63 ± 4.20 vs. Cg: 61.11 ± 1.76, $P<0.001$). No significant reduction in the neutrophil infiltration (58.30 ± 5.60, $P = 0.568$) (Fig. 1 D) was observed for the group 3 points.

NO

The concentration of nitric oxide (NO) in the pleural cavity (Fig. 2A) presented a significant reduction in the groups irradiated with laser when compared to the carrageenan group (1 point: 49.40 ± 2.53 nmol; 3 points: 55.25 ± 2.49 nmol; 7 points: 49.44 ± 2.22 nmol and 14 points: 55.70 ± 2.88 nmol vs. Cg: 86.02 ± 3.86 nmol, $P = 0.001$). It is worthwhile to mention that in laser groups with 1 and 7 points the reduction approximates the basal level values of the saline group (49.78 ± 5.63 nmol).

IL-6

In relation to the pro-inflammatory cytokine, a reduction was observed in the concentration of IL-6 in the irradiated groups after the application of energy when compared to the one that received only carrageenan (1 point: 7298.82 ± 178.99 pg, 3 points: 8596.92 ± 231.76 pg, 7 points: 7955.80 ± 478.74 pg and 14 points: 14587.74 ± 2613.69 pg vs. Cg: 25357.77 ± 5451.93 pg, $P<0.001$). This important mediator presented sensibility to LLLT irradiation independently of the chosen treatment method and energy dose (Fig. 2B).

MCP-1

The chemokine MCP-1 had a significant decrease in laser groups with 1 point (1131.25 ± 175.74 pg, $P = 0.019$), 3 points ($1,228 \pm 209.39$ pg, $P = 0.025$) and 7 points

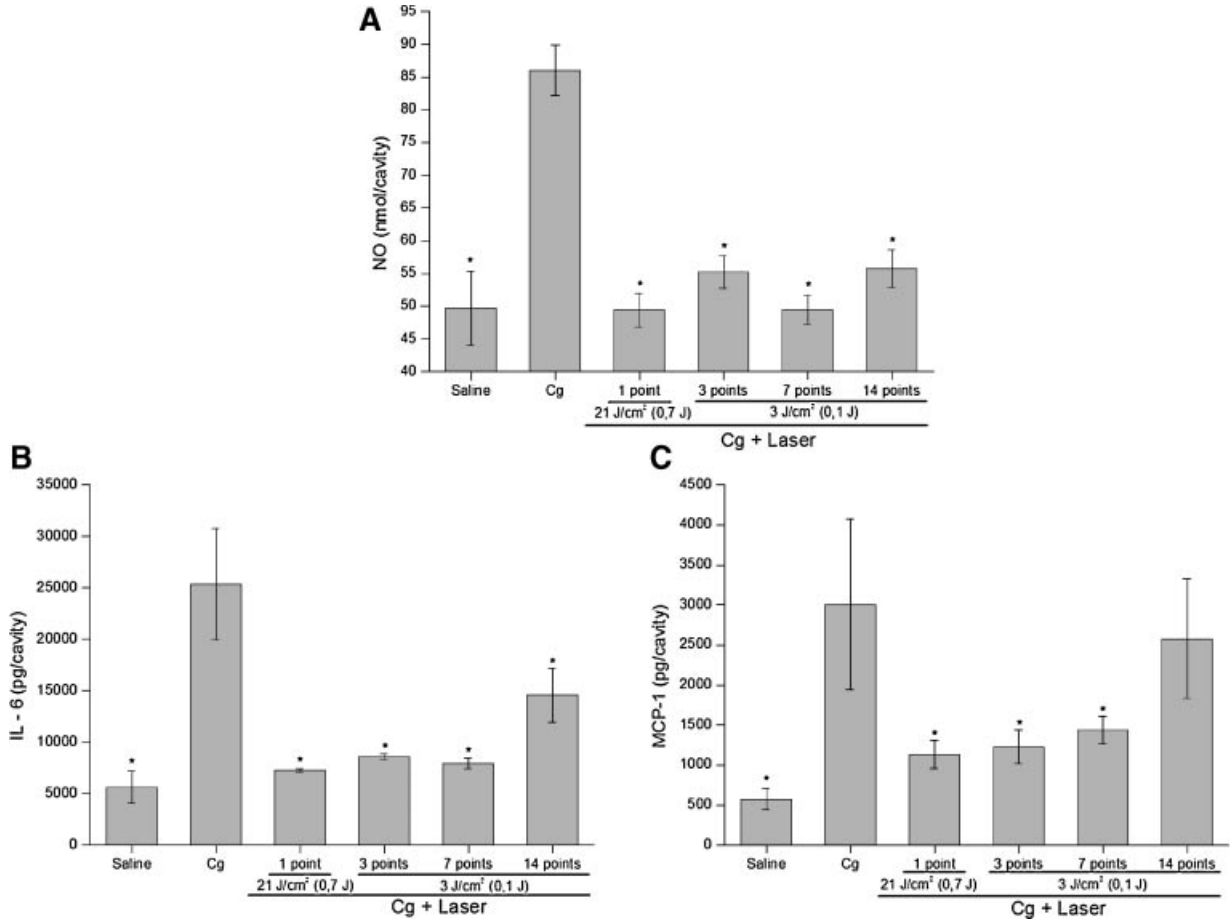

Fig. 2. Effect of low-power laser on the nitric oxide (NO) (**A**), interleukin (IL)-6 (**B**) and monocyte chemoattractant protein-1 (MCP-1) (**C**) that accumulated in pleural cavity after 4 hours of carrageenan injection on the saline, carrageenan (Cg) and laser groups. The Cg+laser groups were irradiated at 1, 2, and 3 hours after induction of the pleurisy with 3 or 21 J/cm^2. The groups with 3 J/cm^2 received it at 3 points (9 J/cm^2), 7 points (21 J/cm^2), 14 points (42 J/cm^2) per session and the group with 21 J/cm^2 received it at 1 point per session. Results are expressed as means ± SEM of eight animals. *$P<0.05$ versus carrageenan group.

(1434.50 ± 171.89 pg, $P = 0.046$) when compared to the carrageenan group (3007.25 ± 1067.61 pg), but in the laser group with 14 points (2578.85 ± 741.09 pg, $P = 0.591$), where the total final energy was equal to 4.2 J, no significant reduction was verified in the measured values from the pleural liquid count (Fig. 2C).

IL-10

A significant reduction was verified of IL-10 concentration in the irradiated groups with 1 point (560.05 ± 70.18 pg, $P = 0.002$), 3 points (690.76 ± 114.44 pg, $P = 0.007$), and 7 points (855.87 ± 239.78 pg $P = 0.049$) when compared to the carrageenan group (1436.15 ± 305.68 pg). In the group with 14 points (957.42 ± 216.25 pg), IL-10 did not show any improvement on the measured values showing that a more local treatment pattern tends to intervene in the final result ($P = 0.088$) (Fig. 3A).

TNFα

The concentration of TNF-α (Fig. 3B) decreased significantly in the LLLT irradiated groups with 1 point (523.90 ± 122.21 pg), 7 points (298.05 ± 69.21 pg), and 14 points (509.05 ± 146.07 pg) when compared to the carrageenan group (1535.20 ± 402.81 pg, $P<0.001$). The laser group with 3 points did not present a decrease in TNF-α levels when compared to the carrageenan group (1206.27 ± 359.99 pg vs. 1535.20 ± 402.81 pg, $P = 0.336$).

Correlation Between Neutrophil Cell Migration With the Study Variables

At 4 hours after carrageenan-induced pleurisy a significant increase was observed in neutrophil migration to the pleural cavity in the carrageenan group when compared to the saline group (Fig. 1D). With regard to all variables correlated to the neutrophil infiltration in the pleural

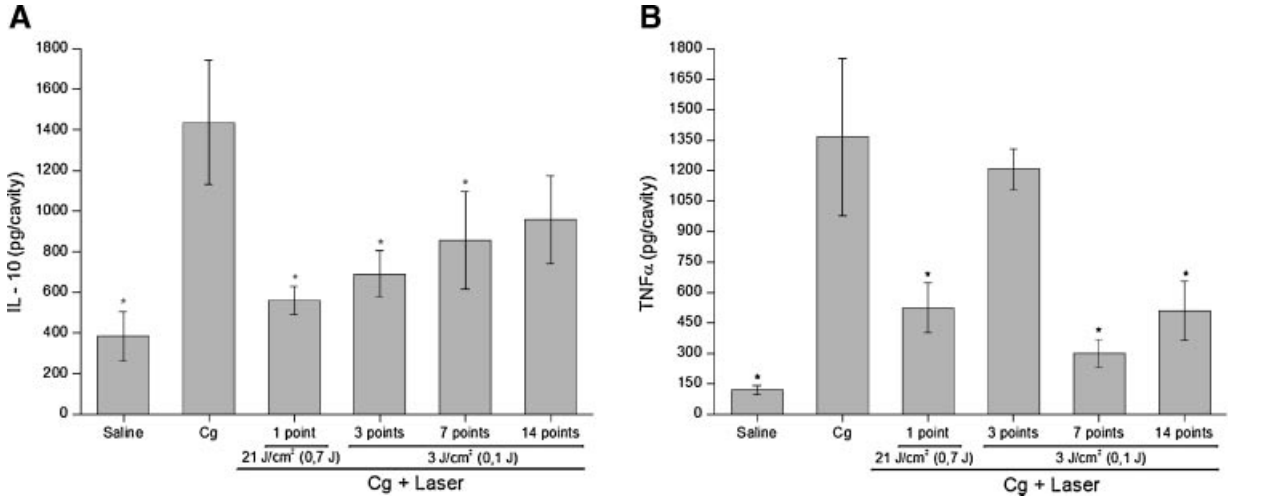

Fig. 3. Effect of low-power laser on the interleukin (IL)-10 (**A**) and tumor necrosis factor (TNFα) (**B**) that accumulated in pleural cavity after 4 hours of carrageenan injection on the saline, carrageenan (Cg) and laser groups. The Cg+laser groups were irradiated at 1, 2, and 3 hours after induction of the pleurisy with 3 or 21 J/cm^2. The groups with 3 J/cm^2 received it at 3 points (9 J/cm^2), 7 points (21 J/cm^2), 14 points (42 J/cm^2) per session and the group with 21 J/cm^2 received it at 1 point per session. Results are expressed as means ± SEM of eight animals. *$P<0.05$ versus carrageenan group.

cavity, only MCP-1 ($P = 0.060$) and IL-10 ($P = 0.092$) did not present a significant statistics-level. In our experimental model, the first inflammatory mediator to show a main correlation with neutrophil infiltration was TNF-α ($r = 0.551$) and the second was NO ($r = 0.549$). The results are summarized in Table 2.

DISCUSSION

Immediately after an acute injury the body initiates a series of biological responses. The inflammatory reaction consists of both vascular and cellular events. Injury responsive components such as mast cells, bradykinins and prostaglandins are activated along with the vascular responses and cellular membrane reactions. All of these combined processes and events are represented by the symptoms of edema, inflammation, pain and functional debility [4,11,27].

The carrageenan is a polysaccharide frequently used to induce acute inflammatory reaction in animal experimental models. Winter et al. [28] introduced the use of carrageeenan as an irritant agent of rat paw edema, becoming the first and most popular method to evaluate new anti-inflammatory therapies with a hydroplethysmometer for inflammated paw volume measurement.

The paw edema induced by carrageenan is an useful method to evaluate acute inflammation, since edema peak occurs within 3–5 hours [29]. However, this technique has some limitations on measuring inflammatory cells, proteins and chemical mediators, once it is not possible to extract the inflammatory exudate.

Despite this, carrageenan-induced pleurisy in rats permits quantifying the volume and protein concentration of the exudate formed, besides the evaluation of the inflammatory cell migration to the pleural cavity [30]. This kind of pleurisy is used to investigate acute inflammation pathophysiology and also to evaluate anti-inflammatory therapies efficacy.

The inflammatory response that occurs after carrageenan injection into the pleural cavity is characterized by cellular infiltration, mainly composed of neutrophils (approx. 90%) and to a lesser extent of monocyte/macrophages [27, 31].

Many studies have suggested advantages of the biomodulatory effects of LLLT on the inflammatory process, wound healing and pain relief. LLLT has been shown to reduce the duration of acute inflammation and pain, just as

TABLE 2. Correlation Between Neutrophil Cell Migration With the Study Variables

	Polymorphonuclear leukocytes (PMNs)	
Variable	Level of significance (P)	Pearson correlation (r)
Total leukocytes	0.001	0.940
Total proteins	0.001	0.845
Pleural exudates	0.001	0.818
TNF-α	0.001	0.551
Nitric oxide	0.001	0.549
IL-6	0.008	0.395
MCP-1	0.060	0.286
IL-10	0.092	0.257

The correlation between the variables and the polymorphonuclear leukocytes infiltration into the pleural cavity in the carrageenan group. TNF-α, tumor necrosis factor alfa; IL-6, interleukin 6; IL-10, interleukin 10; MCP-1, Monocyte chemoattractant protein-1.

to accelerate tissue repair in tendon and muscle injuries [32].

Laser therapy uses different wavelengths of the visible and near-infrared spectra including HeNe (632.8 nm), InGaAlP (630–685 nm), GaAlAs (780–870 nm), and GaAs (904 nm) [33,34]. Many researchers have attempted to understand the action of LLLT, as well as to determine the most appropriate wavelength, period of irradiation, number of treatments, energy density and energy total.

Albertini et al. have demonstrated that LLLT with InGaAlP (650 nm) employed with a continuous power of 2.5 mW and energy density of 2.5 J/cm^2 can reduce edema caused by carrageenan-inflammation in rat paw. In the anti-inflammatory response it has shown similar performance to the sodium diclophenac when intraperitoneally administrated with a dose of 1 mg/kg. In this study the authors verified that LLLT did not inhibit the carrageenan-induced edema in adrenalectomized rat models, suggesting that the mechanism of action can be related with the hypothalamo-hypophyseal-adrenal endocrine system and, as a consequence, with the release of endogenous corticoids [20].

Recently, Lopes-Martins et al. described an inhibitory effect of LLLT (650 nm–2.5 mW) on leukocyte (neutrophil) migration to the pleural space in the carrageenan-induced pleurisy in mice with energy density of 1, 2.5, and 5 J/cm^2, applied on the 1st, 2nd, and 3rd hour after the inflammatory induction. It was observed that the total dose of 7.5 J/cm^2 (0.6 J) was more effective [21].

Based on these studies that have evaluated the anti-inflammatory action of LLLT, the dosage of 2.5 J/cm^2 with power of 2.5 mW has demonstrated to reduce edema and inflamed cells when local irradiation is applied on the inflammated site at 1, 2, and 3 hours after the carrageenan induction, knowing to be dealing with experimental models in which only one point of laser would be sufficient to cover the rat paw or the mouse thoracic wall.

In our study a rat pleurisy model was used but we had no references about points quantity needed to irradiate the most thoracic wall of these animals. Thus, to accomplish our study, we used a continuous-wave power of 20 mW at energy dose of 3 J/cm^2 in three groups with eight animals. These groups were irradiated at 3, 7, and 14 points distributed unequally (14 points symmetrically distributed within the rat's thoracic wall; 7 points and 3 points located on the pleurisy induction site as described above in methods).

The analysis of cell counts (Fig. 1D) showed a more significant decrease of neutrophil infiltration to the pleural space in the group irradiated at 7 points with 3 J/cm^2 in relation to the groups irradiated at 3 points and 14 points. Because our equipment presents a spot of 0.035cm^2 and the dose used was 3 J/cm^2, we concluded that each treatment point corresponds to 0.1 J and, as the total number of applications on the thoracic wall was 7 points, the final energy was 0.7 J. After that, we applied irradiation of 0.7 J at 1 point on the inflammation spot, adding an experimental group to the sample which received a local treatment of 21 J/cm^2.

The energy of 0.7 J applied at 1 point (21 J/cm^2) showed similar results than at 7 points (3 J/cm^2) applied surrounded to the pleurisy-induced site. A significant disparity was noticed in the exudate volume in the group irradiated at 1 point with local treatment when compared to the laser group with 7 points in which the final energy dose was exactly the same. Thus, the local application of energy on the inflammatory process seems to have a better effect on reducing the exudate volume instead of spreading it. The mechanism of this process is still unclear.

The transmigration of neutrophils to injured tissues is a precocious phenomenon of the repair process. It occurs right after the signalization activity mediated by congregated neutrophils. Cytokines (TNF-α, IL-1) act on the endothelial cell receptors inducing NO and cytokines production and cellular adhesion molecule expression in neutrophils.

The quantity of TNF-α (Fig. 3B) in the pleural cavity of the 3 points group demonstrated a similarity between this study and the findings on protein concentration (Fig. 1A), total leukocytes (Fig. 1C) and neutrophils (Fig. 1D), where we did not find a significant variation in the parameters in low doses of LLLT energy. This result gives a dose-dependent effect to this experimental model based on the quantity of energy deposited over the inflamed tissue. Probably the results observed in this group could be related to the fact that LLLT application was done around to the carrageenan injection point.

Shinomiya et al. reported earlier that TNF-α, IL-1, IL-6 were sequentially produced in the exudates of rats with carrageenin-induced pleurisy during the early stage of pleurisy. These chemokines in the inflammatory site cause further chemotaxis to attract granulocytes and monocytes. And the migration of leukocytes, in turn, produces further cytokines and other mediators [35].

The generation of TNFα, IL-6 and MCP-1 in the pleural exudates during the rapid increase in exudate volumes and leukocyte number suggest the involvement of these cytokines in triggering the inflammatory reaction and causing the subsequent responses such as neutrophil infiltration [6]. The cell types responsible for the production of these cytokines are probably both resident cells, mainly monocytes, and migrated neutrophils, because these cytokine levels were rapidly increased almost in parallel with the marked neutrophil influx [7], evincing in our study a moderate correlation between TNF-α and neutrophil infiltration (Table 2).

Whether LLLT can modulate TNF-α in different animal models has been unclear, as previous studies did not find a reduction in TNF-α levels after high doses of LLLT (0.22 J) [18]. Our study shows that LLLT reduces TNF-α levels with total energy dose higher than 2.1 J, contradicting the findings of Aimbire et al. [18].

Non-steroidal anti-inflammatory drugs have been used as remedies for these inflammatory diseases, but several reports have warned that indomethacin increases the production of pro-inflammatory cytokines, such as TNF-α and IL-1, but suppresses IL-6 and IL-8 in the inflammatory

exudates, and that the addition of PGE_2 or PGI_2 reverse these effects [35].

Recent studies, such as Bjordal et al. [36] which investigated in situ if LLLT has an anti-inflammatory effect on activated tendinitis of the human Achiles tendon, concluded that PGE_2 concentrations were significantly reduced after active LLLT compared to concentrations before treatment. Albertini et al. [23] verified recently that LLLT with a wavelength of either 660 or 684 nm (30 Mw–7.5J/cm^2) diminished the formation of edema and the expression of COX-2 mRNA decreased in the subplantar muscle of rat paw subjected to carrageenan-induced inflammation, suggesting a subsequent reduction in production of PGE_2.

Some studies suggest that PGE_2 regulates the cytokine production, such as IL-10, which then suppresses TNF-α production, while another study argues there is no involvement of IL-10 in the inhibition of TNF-α production [35]. In this research we did not evaluate the concentration of PGE_2, but we did not find any correlation between IL-10 and TNF-α and hence the action of laser does not seem to involve IL-10.

Karu et al. [37] investigated the possible regulatory role of NO in the mechanism of cell attachment after LLLT and proposed the existence of signaling pathways between cell mitochondria, the plasma membrane and the nucleus that are activated by visible to near-infrared radiation. Sakaguchi et al. demonstrated that the non-selective NO inhibitor shows an anti-inflammatory effect and whether the combination of an NOS inhibitor and COX inhibitor exerts a synergistic anti-inflammatory effect on acute inflammation such as rat carrageenan-induced pleurisy. The combination of NOS and COX inhibitors showed greater decrease of the exudate volume (43%), leukocyte infiltration (31%) and exudate NO_x level (37%). In our study we achieved a reduction of 67% in the exudate volume, 50% in the leukocyte migration and 42% in the NOx of the pleural exudate after the application of LLLT at one point with 21 J/cm^2. It is likely that LLLT causes the inhibition of NO production, resulting in the potentiation of anti-inflammatory effects with greater results than the combination of NOS and COX inhibitors.

CONCLUSION

We observed a distinct dose–response pattern for the anti-inflammatory effects of LLLT, which were number of points, dose, and total energy delivered in rat pleurisy model induced by carrageenan. The quantity of energy seems to be a more important parameter than the number of points irradiated with laser. Therefore, the healing of the wound in response to local application of LLLT could be a relevant mechanism which contributes to the results observed in the present report. Moreover, any treatment pattern and quantity of luminous energy can reduce an important volume of the exudate to the pleural cavity, but the local application of energy is more efficient than dividing it around the inflammation site.

The LLLT irradiation with total energy lower than 2.1 J reduced only NO, IL-6, MCP-1, and IL-10, while an energy of 2.1 J reduced all variables analyzed in this study, independently of the treatment pattern. The neutrophil migration had a straight correlation with the TNF-α and NO concentration released in the inflammation site, evidencing that the mechanism of the anti-inflammatory action of the laser irradiation can be through the TNF-α or NO. Our results confirm the anti-inflammatory effects of LLLT suggesting it as a clinical alternative to anti-inflammatory drugs. However, many questions regarding molecular and cellular mechanisms by which the cytokines exert these effects after LLLT irradiation remain to be answered.

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