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ORIGINAL ARTICLE

Prevention and treatment of mice paw edema by near-infrared low-level laser therapy on lymph nodes

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Abstract Low-level laser therapy (LLLT) has been demonstrated to modulate inflammatory processes and immunological responses. The aim of this work was to investigate the hypothesis that near infrared LLLT (830 nm) over lymph nodes may reduce paw edema and contribute to the modulation of inflammation. The edema was induced by carrageenan inoculation (*CGN*) into the plantar surface of 100 male mice left hind paw. Animals were divided into five groups: *CGN* (control), no treatment; *Diclo*, sodium diclofenac; *Paw*, LLLT on the paw; *Ly*, LLLT on the inguinal lymph nodes; and *Paw*+*Ly*, LLLT in both paw and lymph nodes, and subdivided according to moment of irradiation: *A*—1 h and 2 h before CGN, *B*—1 h and immediately before CGN, *C*—1 and 2 h after CGN, and *D*—3.5 and 4.5 h after CGN. The parameters used were: energy=1 J, fluence=35 J/cm^2, power=100 mW during 10 s. Paw volume was measured before and 1 to 6 h after CGN, and myeloperoxidase (MPO) activity was analyzed. Edema prevention was obtained by the irradiation of *Paw*+*Ly* at moment A and at *Ly* at moment B, inhibition of edema formation was achieved by either *Paw* or *Ly* at moment C, and edema treatment was obtained by *Paw* or *Ly* at moment D ($p<0.05$). MPO activity was significantly reduced on *Paw* at moment A, *Paw* and *Ly* on C, and in all irradiated groups on B and D. Our results suggest that LLLT was able to produce both anti-inflammatory and pro-inflammatory effects depending on to the site and moment of irradiation.

Keywords Carrageenan · Laser dosimetry · LLLT · Immunological response · Inflammatory process prevention

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Introduction

Inflammatory edema represents one of the cardinal signs of inflammation present in several diseases, such as pharyngitis, tendinitis, or pancreatitis, after trauma and infections, and it is characterized by an accumulation of fluid beneath the skin or in one or more cavities of the body. (Tables 1, 2 and 3)

Edema is one of the most important patients' clinical complaints and is determined by changes in vascular permeability induced by inflammatory mediators or a failure of the lymphatic system. Edema can be life threatening if it results in airway obstruction like it can be seen in the post operative of severe traumas, and orthognathic and head and neck surgeries [1].

The prevention and treatment of edema are very important to avoid discomfort and improve life quality of patients

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Table 1 Experimental groups according to the irradiation moment

Moment A	Laser irradiation performed 2 and 1 h before CGN.
Moment B	Laser irradiation performed 1 h and immediately before CGN.
Moment C	Laser irradiation performed 1 and 2 h after CGN.
Moment D	Laser irradiation performed 3.5 and 4.5 h after CGN.

undergoing surgery or suffering from a disease that causes edema, such as lymphedema installed after mastectomy. Several pharmacological agents have been used, especially corticosteroids [1] and non-steroidal anti-inflammatory drugs (NSAIDs) in order to control edema formation. However, corticosteroids have the potential to develop serious long-term side effects, such as avascular osteonecrosis [2], adrenal suppression [3], decreased healing potential [4], a higher infection rate [5], and steroid-induced psychosis [6], so their prescription have been discouraged. In addition, NSAIDs sometimes cannot be tolerated by the patients due to the high incidence of serious upper gastrointestinal side effects [7]. Therefore, an alternative therapeutic approach with no or less side effects is undoubtedly advantageous.

Low-level laser therapy (LLLT) has been demonstrated to modulate the inflammatory process and the immunological response [8]. This therapy can normalize or reduce the inflammatory responses like edema and inflammatory cells migration [9–14], and has been used in some clinical trials for the treatment of postmastectomy lymphedema. In fact, irradiation with low power lasers to the axillary region can assist in resolving postmastectomy lymphedema (PML) by reducing fibrosis caused by breast cancer related to intervention [15, 16]. In addition, it can also stimulate the generation of surviving lymphatic drainage pathways, and activating the localized immune response [17].

Laser irradiation directly over lymph nodes could promote beneficial effects in controlling edema since the lymphatic system is directly involved in edema resolution and lymph nodes on the inflammatory process is one of the most important sites for immune cells to exchange information, reside, expand, and initiate adaptive immune responses. However, controversy exists regarding the mechanisms that are involved in the modulation of inflammation when the laser is used.

Table 3 Area under the curve (±SEM) and % (percentage) of edema reduction (±SEM) of moments A, B, C, and D during experimental period (6 h)

	Group	Área under de curve	(± SEM)	% Reduction	(± SEM)
Moment A	CGN	2.86	0.,18		
	Diclo	1.18*	0.10	58.7	3.8
	Ly	2.67	0.17	6.9	6.4
	Paw	2.88	0.21	−0.6	7.9
	Paw+Ly	2.06*	0.15	28	5.8
Moment B	CGN	2.86	0.18		
	Diclo	1.18*	0.10	58.7	3.8
	Ly	1.58*	0.14	44.9	5.3
	Paw	3.51	0.30	−23	11
	Paw+Ly	2.69	0.30	6	11
Moment C	CGN	2.86	0.15		
	Diclo	1.18*	0.09	58.7	3.3
	Ly	1.57*	0.10	45.2	3.9
	Paw	1.28*	0.13	55.2	4.8
	Paw+Ly	2.18	0.13	23.7	5.1
Moment D	CGN	2.86	0.18		
	Diclo	1.18*	0.10	58.7	3.7
	Ly	1.98*	0.17	30.8	6.3
	Paw	2.06*	0.14	28.0	5.4
	Paw+Ly	2.86	0.37	0	13

N=27 for CGN, N=13 for DICLO, and N=5 for all test groups of each moment

*p<0.05, statistically significant differences compared to CGN group

Some studies suggest that LLLT can minimize inflammatory reactions [18] while others believe that this therapy may exacerbate it [19, 20], which could result in more or less edema formation. Probably, the type of response might be related to the phase of inflammatory process and edema formation present in the tissue when laser irradiation is performed. Moreover, LLLT may have a role in immunobiological therapy for diseases of the immune system and may activate and increase normal reaction of the immune system components [21].

In this study, we hypothesize that LLLT may produce a positive effect on acute inflammatory edema depending on the moment and site of irradiation and that a prophylactic

Table 2 Description of the five experimental groups according to the treatment carried out

CGN	Control group	CGN injection at 0 h (edema induction) solely
Diclo	Sodium diclofenac treatment group	1 mg/kg of sodium diclofenac was injected 30 min before edema induction
Paw	Paw group	CGN injection (0 h)+laser treatment in the middle of the left hind paw following respective moments of irradiation
Ly	Lymph node group	CGN injection (0 h)+laser treatment in the left inguinal lymph node following respective moments of irradiation
Paw+Ly	Paw and lymph node group	CGN injection (0 h)+laser treatment in the paw and in the left inguinal lymph node following respective moments of irradiation

irradiation may contribute to the modulation of inflammation. For this purpose, we evaluated the effects promoted by LLLT when the irradiation is applied before, during and after edema formation as well as the influence of systemic (lymph nodes) or local irradiation on acute inflammatory response in mouse paws induced by carrageenan (CGN) [22].

Material and methods

Animals

One hundred Swiss male adult mice (40 days old) were kept in standard laboratory conditions (20–22 °C, 10–14 h light–dark cycle) supplied with food and water ad libitum in accordance with the guidelines of IPEN/CNEN for animal care and approved by the ethics committee (CEPA-IPEN/SP N.21/2008).

Carrageenan-induced edema

The inflammatory edema was induced by subcutaneous injection of 50 μL of 1 % CGN suspended in sterile saline solution (Sigma Chemical Co., St. Luis, MO, USA; 0.5 mg/paw) into the plantar surface of the left posterior mouse paw. Paw volume was measured using a water displacement plethysmometer (Plethysmometer 7150, Ugo Basile, Italy) immediately before and 1 to 6 h after CGN injection. The increase in paw volume (mL) was calculated by subtracting the basal volume (measured just prior to CGN injection) from the final volume of each time point. After 6 h, the animals were killed by cervical dislocation and the tissue from the plantar surface was carefully removed and immediately frozen in liquid nitrogen, stored at −80 °C until myeloperoxidase (MPO) activity assay was performed.

Laser irradiation

A continuous diode infrared laser (GaAlAs; Thera Lase DMC, São Carlos—SP, Brazil), emitting at $\lambda = 810$ nm with an output power of 100 mW was used. The sites of laser irradiation were the middle of the left hind paw, the left inguinal lymph node [23] or both, depending on the respective experimental group. In all groups, two irradiations of 1 h time interval were performed and the laser tip distance from the skin was adjusted using a 2-mm spacer (irradiation area of 0.028 cm^2), during 10 s, corresponding to 1 J of delivered energy per site, fluence of 35.7 J/cm^2, and power density of 3.5 W/cm^2.

Myeloperoxidase (MPO) activity

Myeloperoxidade is a specific marker of neutrophils and MPO assay indicates the activity of this protein. The method of measurement of MPO activity is based on the speed of oxidation of *o*-dionisidina in the presence of hydrogen peroxide, as evidenced by the change of absorbance measured by spectrophotometry. The frozen samples were homogenized in 1 mL of potassium phosphate buffer (50 mM, pH 6.0) containing 0.5 % of hexadecyltrimethylammonium bromide (Sigma, USA) and 5 mM of EDTA, pH 6.0. The homogenized samples were heated (2 h, 60 °C) and then centrifuged at 12.000×*g* for 2 min at 4 °C. The supernatants were mixed with phosphate buffer containing *o*-dionisidine dihydrochloride (0.164 mg/mL, Sigma, USA) and 0.0005 % hydrogen peroxide (Merck, Germany). Absorbance was evaluated at 460 nm using a spectrophotometer (SpectraMax plus 384, USA), during 10 min. MPO activity was expressed as UMPO (MPO unit) what is the amount of MPO in hydrogen peroxide (μM) degraded per minute.

Experimental groups

Four experiments of 25 animals were performed varying the irradiation moment A, B, C, and D (Table 1) as much as one of the purposes of this work is to test different moments of irradiation in order to verify the action of LLLT during all phases of inflammatory process. The animals of each experiment were randomly divided into five subgroups ($N=5$) according to the treatment realized (*CGN*, *Diclo*, *Paw*, *Ly*, *Paw+Ly*; Table 2).

Data from all CGN ($N=27$) and Diclo ($N=13$) subgroups pooled after ANOVA and Levene tests shown that there was no evidence of statistically significant difference between the means and variances of these groups ($p>0.05$). Diclo group had a homogeneous pattern of results being required fewer animals than CGN.

Statistical analysis

The data obtained were normalized and statistically analyzed using analysis of variance (ANOVA) at 5 % followed by Tukey test. Data are expressed as means±SEM (standard error of the mean). The results were considered significant when $p<0.05$.

Results

Edema evaluation

Diclo and *CGN* groups were statistically grouped generating a standard profile used in all four experiments. The *CGN* group of all experiments showed an edema evolution pattern with a peak between the third and fourth hours. At this time, edema volume was increased by 65 % compared to basal volume and then it was decreased by 30 % after 5 h. *Diclo* group also

showed a pattern already expected with inhibition of edema along whole experimental time (6 h), since the animals were treated 30 min before CGN injection (Figs. 1, 2, 3, and 4).

Moment A

On moment A, the irradiation was delivered in a preventive manner, 2 and 1 h before edema induction. The results show the gradual decrease in the edema of *Paw*+*Ly* group starting on the second hour and being statistically lower than all irradiated groups on the fourth hour. *Ly* group reached its pick on the third hour and then showed a significant drop, with swelling below the *CGN* group on the fourth hour. *Paw* group showed statistically similar volume to *CGN* group in 4 h, despite having followed the same evolution curve of *Ly* group (Fig. 1).

In order to analyze which laser irradiation group performed the best treatment to prevent the edema, the area under the curve was performed. *Paw*+Ly group was the best irradiation option when applied 2 and 1 h before edema induction, being the only irradiated group capable of inhibiting the swelling when compared to the *CGN* group, reducing the edema in 28 % (Table 3, moment A). However, if the experiment ended in 4 h, no differences would be found between all irradiated groups and *CGN* group ($p>0.05$).

Moment B

The preventive irradiation performed 1 h and immediately before the edema induction showed some interesting results. The edema was prevented only in Ly irradiated group from baseline until the third experimental moment. During this period, the measured volume was statistically similar to Diclo group and starting from the fourth hour the edema volume of all irradiated groups was similar to *CGN* group (Fig. 2).

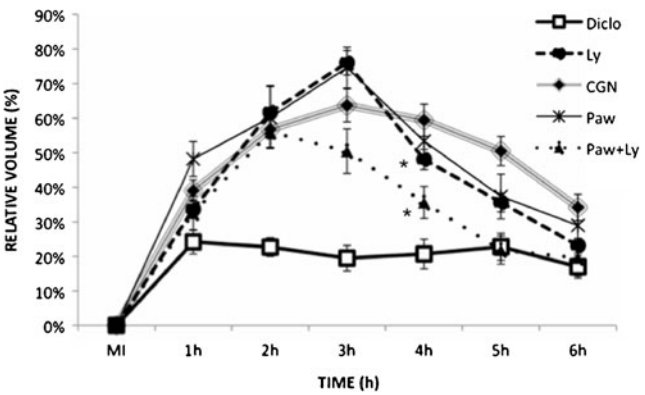

Fig. 1 Average of the volume of edema (%) ± SEM (standard error of mean) of moment A. The irradiation was carried out 2 and 1 h before the injection of carrageenan at initial moment (MI); $N=27$ for CGN group, $N=13$ for Diclo group, and $N=5$ for the other groups. Statistically significant differences compared to CGN are indicated by an *asterisk* ($p<0.05$)

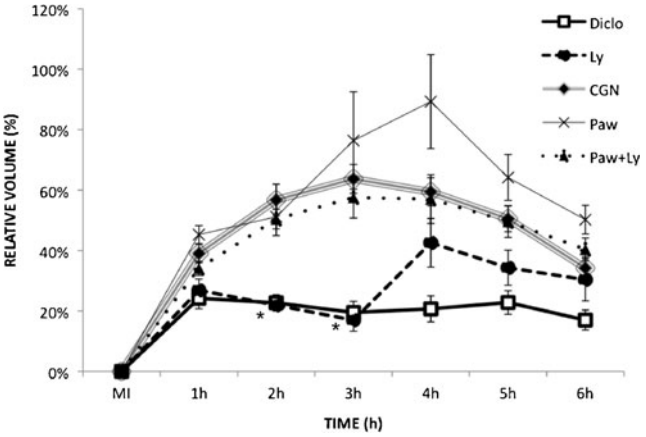

Fig. 2 Average of the volume of edema (%) ± SEM of moment B. The irradiation was carried 1 h and immediately before the injection of carrageenan (MI); $N=27$ for CGN group, $N=13$ for Diclo group, and $N=5$ for the other groups. Statistically significant differences compared to CGN are indicated by an *asterisk* ($p<0.05$)

Although no statistical difference was demonstrated, *Paw*+Ly and *Paw* groups showed a trend throughout the experiment important to be observed. Contrary to the pattern observed on moment A, *Paw*+Ly group had no effect on the development of edema. However, *Paw* group showed an exacerbation of edema. The area under the curve showed that the best group to prevent edema 1 h and immediately before the induction was the Ly group, with an edema reduction of 44.9 % compared with *CGN* group (Table 3, moment B).

Moment C

The irradiation performed 1 and 2 h after edema induction aimed to evaluate the swelling in its upward trajectory. After 2 to 4 h from carrageenan injection, *Paw* and Ly groups

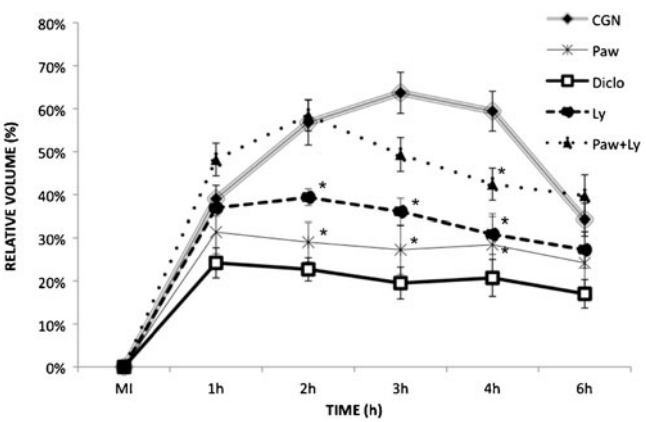

Fig. 3 Average of the volume of edema (%) ± SEM of moment C. The irradiation was carried 1 and 2 h after the injection of carrageenan (MI). *Arrows* represent the irradiations; $N=27$ for CGN group, $N=13$ for Diclo group, and $N=5$ for the other groups. Statistically significant differences compared to CGN are indicated by an *asterisk* ($p<0.05$)

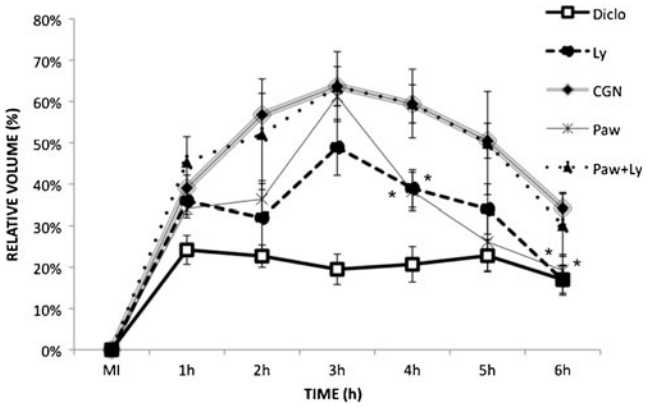

Fig. 4 Average of the volume of edema (%) ± SEM of moment D. The irradiation was carried 3.5 and 4.5 h after the injection of carrageenan (MI); N=27 for *CGN* group, N=13 for Diclo group, and N=5 for the other groups. Statistically significant differences compared to CGN are indicated by an *asterisk* (p<0.05)

were effective in inhibiting edema when compared to the *CGN* group. Although edema levels of *Paw*+*Ly* have shown to decline from the second hour, the edema was statistically lower than the *CGN* group only in 4 h (Fig. 3).

Paw and *Ly* groups proved to be the best treatment options, inhibiting the development of edema in 55.2 % and 45.2 %, respectively, when compared to the *CGN* group along 6 h evaluation of the area under the curve (Table 3, moment C). However, *Paw* group appeared to be even more effective in reducing edema than Ly group, as it has demonstrated similar results to the group treated preventively with sodium diclofenac (*Diclo*). Moreover, evaluating the area under the curve of 4 h, only *Paw* group differed from *CGN* group (p=0.0049). Despite having a drop in volume initiated at 2 h after carrageenan, *Paw*+Ly group showed no advantages compared to *CGN*.

Moment D

Paw edema was at its peak after 3.5 and 4.5 h of edema induction and therefore laser irradiations performed at this time consisted in the treatment of installed edema. At 3 h, laser irradiated groups were similar to *CGN* group, and since treatment with sodium diclofenac was preventive, all groups are also statistically different from this one.

The effects of the first irradiation at 3.5 h can be seen in 4 h, when edema levels of *Paw* and Ly groups were statistically lower than the *CGN* group and similar to Diclo group. Similarly, the effects of second irradiation at 4.5 h could be seen in 6 h with the same results (Fig. 4).

Analyzing the effectiveness of treatments over the whole experimental time (6 h), Ly and *Paw* groups were more effective than *Paw*+Ly group in the treatment of edema when compared to *CGN* group, with a reduction of 30.8 % and 28 %, respectively (Table 3, moment D). In 4 h, however, the effects are related to a single irradiation and therefore have no statistical difference with *CGN* control group (p>0.05).

Mieloperoxidase activity

The mieloperoxidase activity was measured after 6 h from edema induction and represents the neutrophil accumulation in the paw according to the different treatments performed. At moment A, only the *Paw* group showed less neutrophil accumulation than *CGN* group. However, when the irradiations were performed on moment B (1 h and immediately before edema induction) all groups showed less neutrophil accumulation than *CGN* group. At moment C, during the evolution of edema, *Paw*+*Ly* group was similar to *CGN* group, and both showed greater neutrophil infiltration than the other two irradiated groups. The treatment of edema at the moment D, held after the peak of edema, showed a reduction in neutrophil infiltration in all irradiated groups compared with *CGN* group (Fig. 5).

Discussion

Many authors demonstrated some positive effects of laser irradiation in reducing the edema and inflammatory signals [7, 9–11, 24–27]. LLLT is able to activate cells as lymphocytes, to stimulate lymphatic flow and to modulate the inflammatory process [14, 20, 28, 29]. Karu [30] and others authors [31, 32] performed studies in cell culture and have shown that laser effects can only be seen during the state of oxidative stress. However, when animal studies are conducted, it is important to consider the influence of laser irradiation in the immune response, which, together with the inflammatory response, will act on the resolution of pathological processes. Some studies provide convincing evidence of immunostimulative effects of the low power red light [33–35]. Also, near infrared low-level laser irradiation at 904 nm showed systemic immunomodulatory effects, suppressing contact hypersensitivity reaction [36].

Therefore, it is important to look for new therapies that contemplate not only the local of inflammation but also the whole immunological system. Once the lymph nodes are directly involved in the inflammatory process, its irradiation may produce a beneficial effect on the modulation or resolution of inflammation. Moreover, inflammatory process follows a complex sequence of events, and it is important to study what is the right time/moment for irradiation, and what are the laser effects at different stages of the process. The laser may also have preventive effects when cells that can be activated by light are irradiated. Thus, it was proposed to investigate the importance of the laser irradiation in different stages of the inflammatory reaction.

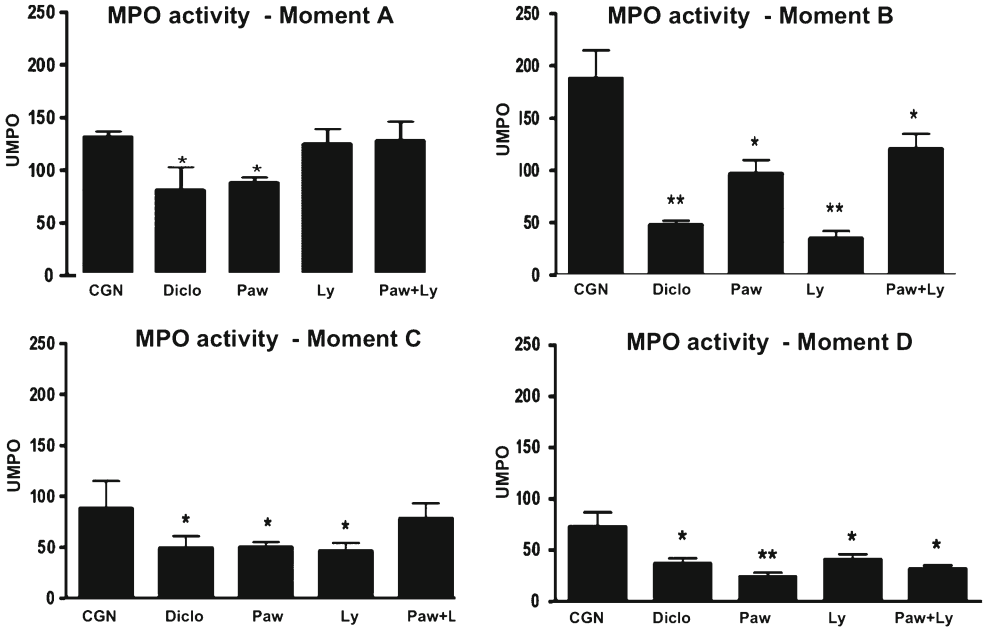

Fig. 5 Mean values ± SD (standard deviation) of units of myeloperoxidase (UMPO) measured 6 h after injection of carrageenan on moments A, B, C, and D. $N=5$ animals per group; $*p<0.05$, $**p<0.01$ compared to GGN group

In this work, we used a power density of 3.5 W/cm^2 and a fluence of 35.7 J/cm^2. These values for power density and fluence are not commonly reported in literature and someone can ask if there was temperature increase into tissue. Indeed, the amount of heat generated by optical illumination of tissue is dependent upon the power density or irradiance (W/cm^2) as well as the wavelength, absorbance of the tissue and the length of time the illumination lasts for. Skin is a highly scattering medium and for near infrared wavelengths forward scattering prevails over absorption so light penetrates deeper. In our methodology, we used 3.5 W/cm^2 during 10 s in non-contact mode to delivery 1 J of energy. We believe that energy is a better descriptor to LLLT since radiometric quantities and units can quantify only the exposure responsible for the initial event—the optical radiation exposure of the target or biological tissue [37]. Besides, since light decays exponentially inside tissue, after 1 mm the power density would be lower than 1 W/cm^2. In fact, studies in our lab showed that the rise in temperature using a power density of 10 W/cm^2 during 3 min is about 1 °C (data not shown). Since in this work we used about 1/3 of the power density of the above mentioned pilot experiment, and 6 % of the time, we may infer that the rise in temperature would be insignificant.

On the other hand, the same protocol of acute inflammatory edema induced by carrageenan was performed [11, 12] and different edema treatments were used. To represent the best conventional treatment, one group received the injection of sodium diclofenac 30 min before the edema induction. We did not perform laser and sodium diclofenac at the same moment, as it was not our goal to find out the best moment for the sodium diclofenac injection, but to study the irradiation best moment, and each protocol tested was considered a different treatment. A control group was performed without any treatment, and the irradiated groups varied according to the moment and site of irradiation.

Interestingly, two different and opposite effects could be found according to irradiation site and moment performed in this study, anti-inflammatory and pro-inflammatory effects. The anti-inflammatory effect seems to inhibit the edema formation without necessarily reducing the infiltrate of inflammatory cells. It was demonstrated a 28 % of reduction in paw swelling in *Paw+Ly* group at moment A compared with *CGN* group, with no effects on MPO analysis.

The pro-inflammatory effect was previous observed by Albertini *et al*., [11] who demonstrated that laser enhanced paw edema when irradiation occurred immediately after carrageenan injection. At moment B, *Paw* and *Paw+Ly* laser groups were irradiated 1 h and immediately before CA injection and were not effective on edema reduction. On the contrary, they stimulated the increase of swelling even though showing significantly inflammatory cell reduction at the end of experiment. The exacerbation of edema found in *Paw* laser group at moment B could be explained by immediately enhancement on local microcirculation [38], contributing to the CGN spread and consequently magnifying toxic effects. The observed pro-inflammatory effect can also be interpreted as an acceleration of the process leading to a faster resolution with the reduction of inflammatory cells at the end of the experiment (6 h after edema induction), but not necessarily inhibiting the swelling. *Paw* group at moment A illustrate it, being the only irradiated group to reduce inflammatory cells 6 h after the edema induction, without inhibiting the edema. In addition, the reversion in edema evolution observed from the third hour, even lately confirms the accelerating effect over the inflammatory response following directly irradiation on paw.

Both effects, pro and anti-inflammatory, however, seem to be equilibrated in groups where inflammatory response was better controlled. *Ly* group at moment B (preventive), and *Paw* and *Ly* groups at moments C and D (treatments) were effective in reducing edema as well as in reducing the inflammatory infiltrate when compared to *CGN* group. Moreover, *Ly* group treated in a preventive manner at moment B, and the *Paw* group at moment C, showed similar edema reduction than the group treated with sodium diclofenac. These results demonstrate that LLLT have similar potential in edema reduction as sodium diclofenac, encouraging its use as an anti-inflammatory alternative treatment, with the additional advantage on not bringing any important side effects.

In order to achieve the best results, it seems to be important to irradiate the lymph nodes if irradiation is close to the moment that the lesion occurred to stimulate the immune system cells to go to the lesion area. On the other hand, to stimulate the removal of local oxygen reactive species and reduce the local inflammatory response is better to irradiate the local of the lesion if the edema already started. Also, the dose of irradiation should be high enough to prevent edema formation as in *Paw+Ly* group at moment A and in Ly group on moment B. To inhibit or treat the edema is important to take into account the dose and location of irradiation. Double irradiation was less effective on edema reduction and an overdose of aversive stimulus could explain it. If Paw+Ly were irradiated immediately before the lesion, the pro-inflammatory effect of paw irradiation may have nullified the anti-inflammatory effect of lymph node irradiation resulting in a null response. However, even with less effective edema reduction, neutrophil accumulation was reduced at moments B and D. At moment B, paw was irradiated immediately before the carrageenan injection and an increased pro-inflammatory pattern was observed. It could explain the lower MPO activity as the inflammatory process was accelerated. At moment D, the irradiations were made shortly before the assessment of MPO activity and the immediately laser effects obtained could be represented by the reduction of inflammatory cells on the mice paws.

The laser irradiation over the lymph nodes contributing to enhance the immunological response in a preventive manner brings a new approach for LLLT. Among all the LLLT proposed mechanisms of action, the effect of laser irradiation on the intracellular calcium increase is well established in the literature [39]. As an important cellular messenger, calcium influx can produce cell stimulation and proliferation. Thus, the laser effects over the ion channel functions could be the mechanisms by which the preventive laser irradiation over the lymph nodes modulates the inflammatory process. Ion channels may play a role in both the activation and modulation of the inflammatory response, as the activation of T-lymphocytes, by the influx of calcium. The opening and closing of ion channels may modulate the movement of some immune system cells to the site of inflammation, the release of chemical signaling factors from immune system cells, and the proliferation of these cells in response to activation of the immune system. However, the exact mechanism of action of the laser effect observed over the lymph nodes remains to be established.

Based on the results found with the experimental model used in this study, the following treatments protocols could be indicated to prevent and treat the edema: preventive laser irradiation at paw and lymph nodes 2 and 1 h before CGN and preventive laser irradiation at lymph nodes 1 h and immediately before CGN. To inhibit edema formation, *Paw* or lymph node irradiation should be performed 1 h and 2 h after CGN, and finally, to treat an installed edema, the *Paw* or lymph node irradiation should be performed 3.5 and 4.5 h after CGN.

However, the importance of the edema reversion at any time during the inflammatory process should be considered. The immediate laser effects could be seen after each irradiation at moments C and D when the *Paw* or lymph nodes were irradiated and, also, after *Paw+Ly* laser irradiated group at moment C. So, at any time during the evolution of the inflammatory response, the irradiation could punctually act reducing discomfort and pain of experimental animals, or if we consider the clinical situation at any time the irradiation would reduce the edema and improve the quality of life of patients.

In conclusion, laser irradiation showed an anti-inflammatory and a pro-inflammatory effect according to the site and moment of irradiation. Near infrared low-level laser therapy showed to be efficient on edema prevention and treatment when paw, lymph nodes, or both were irradiated. In this context, we herein confirm that LLLT can be a potential alternative to anti-inflammatory drugs on edema treatment and prevention.

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