Trends in Photochemistry & Photobiology
Vol. 15, 2013

Original Communication

Effect of Helium-Neon (HeNe) laser irradiation on lesions in experimental paracoccidioidomycosis

Thiago Alves da Costa[1], Carolina Francelin[1], Rosária Di Gangi[1], Maria Renata Sales Nogueira Costa[2], Rodolfo Thomé[1], Luciana Campos Paulino[3] and Liana Verinaud[1,*]

[1]Department of Structural and Functional Biology, Institute of Biology, University of Campinas - UNICAMP, Campinas, São Paulo, Brazil, [2]Lauro de Souza Lima Institute, Bauru, São Paulo, Brazil, [3]Center of Natural and Human Sciences, Federal University of ABC- UFABC, Santo André, São Paulo, Brazil

ABSTRACT

Cutaneous lesions caused by the fungus *Paracoccidioides brasiliensis* lead to ulcerative wounds, which are difficult to be cured by conventional treatment with anti-fungal drugs due to their adverse effects and the multidrug resistance that some fungal isolates present. So, the laser therapy emerges as an alternative treatment since its anti-inflammatory effects and wound healing properties are already known. In this work, we showed that lesions caused by the inoculation of yeast cells into the back foot-pad of BALB/c mice and treated with HeNe laser present greater histologic organization, milder inflammatory infiltrate, higher organization of the granulation tissue around the lesion, and enhanced immunolabeling for iNOS. In addition, an increased SOD activity and NO concentration, higher percentage of macrophages, and lower neutrophil numbers were observed. The percentage of NK and NKT cells were also slightly higher after the first laser session. Altogether these results point towards a dual effect of the laser treatment, decreasing the inflammatory response and accelerating the wound healing of the lesions. In this sense, the HeNe laser can be considered as an effective adjunctive treatment to be combined with pharmacologic therapies for improving the treatment of painful non-healing paracoccidioidomycotic wounds.

*Corresponding author: verinaud@unicamp.br

KEYWORDS: low-level laser therapy, HeNe laser, skin lesions, inflammatory response, paracoccidioidomycosis, *Paracoccidioides brasiliensis*

INTRODUCTION

The thermodimorphic human pathogenic fungus *Paracoccidioides brasiliensis* (*Pb*) is the etiological agent of the most important systemic mycosis in Latin America, the paracoccidioidomycosis (PCM) [1]. The infection starts in the lung, due to the inhalation of infecting propagules, but has a tendency to disseminate to other tissues or organs via lymphatic and/or blood vessels [2-5]. In the skin and mucous membranes the infection leads to painful and sensitive lesions where the fungi remain fully pathogenic and active for a long period even after the beginning of pharmacologic therapy. Furthermore, it is not uncommon that mucocutaneous lesions progress to ulcerative wounds that predispose the host to secondary microbial infection [6, 7].

Cutaneous lesions in PCM are conventionally treated with systemic antifungal agents, which include sulfonamides, amphotericin B, or imidazoles, but very few patients achieve full remission from treatment with these medications either when

given alone or in combination. Besides, such therapies induce systemic adverse effects to the patients, are long-term treatments, and have their efficacy frequently questioned due to the multidrug resistance that some *Pb* isolates present [8]. So, the search for non-pharmacologic therapies, such as the low-level laser therapy (LLLT), which may also be effective alternative and/or adjunctive treatments for maximizing patient healing with avoidance of toxic effects [9], is necessary, desirable, and justified.

The helium-neon (HeNe) laser has emerged as an alternative treatment since its microbicidal activity and its ability to heal wounds without changing cell function are already well known [10, 11]. Although the exact mechanism of LLLT remains to be established, some reports document anti-inflammatory effects, improvement in tensile strength of wounds, and reduction of painful symptoms through the biostimulation of cellular events [12, 13].

Using an experimental model of paracoccidioidomycotic lesions caused by the inoculation of yeast cells into the back foot-pad of BALB/c mice, our group has explored the biological mechanisms underlying the inflammatory process after three sessions of HeNe laser treatment.

Our results demonstrated that HeNe laser-treated lesions presented reduction in footpad edema, faster cutaneous wound healing, confluent granulomas, diffuse and more loosely-distributed immunolabeling for Tumor Necrosis Factor-alpha (TNF-α), and enhanced labeling of Interferon-gamma (IFN-γ) [14]. In another study, we reported alterations in lesion microenvironment after laser treatment such as inhibition of the CXCL10 chemokine secretion and hypoxia-inducible factor 1 (HIF-1) expression, increase of vascular endothelial growth factor (VEGF) expression, and decrease of reticulin fiber deposition [15]. Finally, in a more recent study, we have also observed decreased levels of pro- and anti-inflammatory cytokines and of chemokine ligand 3 (CCL3), as well as decreased density of fibronectin and laminin fibers in lesions exposed to HeNe laser irradiation [16].

The purpose of this work was to analyze some immune modulatory effects occurring early on the next day after the action of HeNe laser on the healing of paracoccidioidomycotic injuries to support and corroborate the data obtained previously.

MATERIALS AND METHODS

Animals

Specific pathogen free BALB/c male mice, 6-8 week-old, were purchased from Centro Multi-Institucional de Bioterismo (CEMIB) and maintained in transparent acrylic plastic isolators under aseptic conditions throughout the study, with sterile water and feed provided *ad libitum*. All procedures were carried out in accordance with the guidelines proposed by the Brazilian Society on Animal Care and approved by the animal care and use committee of the University of Campinas (protocol 1944-1).

Fungal strain

The virulent isolate Pb18 of *P. brasiliensis* was used throughout the experiments. Fungal cells were grown at 37°C in Fava Netto's medium and used at the 7th day of cell culture growth. Viability was determined by Lactophenol cotton blue staining [17] and was higher than 90%.

Infection of mice

Thirty mice received $5x10^6$ yeast forms of *P. brasiliensis* contained in 50 µl buffer into the left back footpad, followed immediately by 50 µl sterile saline into the opposite one. Animals were monitored daily for the progression of lesions, which peaked at 7 days after inoculation.

Laser treatment

Seven days after *P. brasiliensis* infection, the animals were separated into twelve sets of five mice each (six sets in the experimental group and six sets in the control group). On days 7, 8 and 9 following infection, the lesions of mice from the experimental group were irradiated with laser (referred as T1, T2 and T3, respectively). The control group underwent the same handling, but was sham-irradiated (referred as non-treated animals). The laser used in this study was a helium-neon

laser with a measured output of 3.5 mW, which emits light in a collimated beam (diameter 4 mm) with a wavelength of 632.8 nm. The irradiating flux for each treatment (previously determined by dose response studies) was 3.0 J/cm^2 with a stimulation time of 110 seconds. The laser probe was applied punctually, in contact with the wound, without anesthesia or sedation. Twenty-four hours after each treatment, five animals from each group were killed by CO_2 inhalation and the footpads removed for analysis.

Histopathological analysis

The footpads were collected and fixed in 4% paraformaldehyde solution for 16 hours at 4°C. The specimens were submitted to diafanization with xylene, dehydrated by graded ethanol, embedded in paraffin and cut in 5-µm-thick sections. Histologic changes were evaluated on sections stained with hematoxylin and eosin (H&E).

Flow cytometry analysis of cell populations in paracoccidioidomycotic lesions

After sacrifice, the footpads from laser-treated and non-treated animals were removed, soaked and mashed in phosphate-buffered saline (PBS) containing 5% fetal calf serum. The solution obtained passed by a cell strainer with mesh pore size of 100 µm (BD, San Diego, California, USA) and was then centrifuged at 1000 g for 15 minutes at 4°C for subsequent cellularity evaluation. The red cells were removed by using ammonium chloride lysis buffer (0.16 M NH_4Cl, 0.01 M $KHCO_3$, 0.001 M EDTA, pH 7.4). Multi-color flow cytometric phenotypic analysis was performed with a Gallios device (Beckman Coulter) using fluorescent isothiocyanate (FITC), phycoeyrthrin (PE) and allophycocyanin (APC) as fluorescent dyes. Directly conjugated anti-mouse monoclonal antibodies were used: CD3, CD11b, Ly6G/6C (BD Pharmigen, San Jose, CA, USA), CD11b and NK1.1 (eBioscience, San Diego, CA, USA). For macrophages detection anti-mouse F4/80-like receptor was used with Mouse BD Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2, the staining was amplified by using a biotinylated anti-rat IgG2a mAb RG7/1.30 second-step antibody followed by a "bright" third-step reagent, Streptavidin-APC (BD Pharmigen, San Jose, CA, USA). Fluorochrome-labelled isotype-matched negative controls for the specific monoclonal antibodies were also obtained from Pharmingen. The assessment of neutrophil population was performed using the subtraction of macrophages population from CD11b and Ly6G and Ly6c positive cells. Cells were stained for 20 min and then washed with PBS, fixed with paraformaldehyde 4% and analyzed by flow cytometry in a Gallios device (Beckman Coulter). Analyses were performed after recording 10.000 events for each sample using Flow Jo 7.6.5 (Ashland, OR, USA).

Immunohistochemical analysis

The production of inducible isoform of Nitric Oxide Synthases (iNOS) by macrophages in laser-treated and non-treated lesions was done by using immunohistochemical method. The footpad sections were deparaffinized with xylene, hydrated in an ethanol gradient and water before stain reaction. Directly after, incubation was performed with 3% H_2O_2 in absolute methanol (v/v) to block endogenous peroxidase activity (10 min). From here all procedures were done according with ImmunoCruzTM rabbit ABC Staining System (sc-2018) datasheet. Briefly, all sections were incubated with goat serum to avoid secondary antibody nonspecific binding for one hour at 4ºC and then incubated with specific primary antibodies to mouse iNOS both from AbCam (Cambridge, Cambridgeshire, UK) for 16 hours at 4ºC. After washing, sections were overlaid for 1 h with the secondary antibody biotin-conjugated. This was followed by incubation with AB enzyme reagent to amplification of signal reaction. Bound antibodies were detected by reactivity with 3,3`-diaminobenzidine plus H_2O_2. After tap washing, the slides were counterstained by Harris Haematoxilin and mounted with Permount. For immunohistochemical controls primary antibodies were omitted from the staining procedure and were negative for any reactivity. Quantification of the immunostaining (integrated density of pixels/area) was done by using the Image J software [18].

Assessment of tissue nitric oxide (NO) concentration and superoxide dismutase (SOD) activity

After sacrifice, the footpads from laser-treated and non-treated animals were removed, soaked and

mashed in phosphate-buffered saline (PBS). The solution obtained was filtered with a syringe filter of 0.22 µm and then centrifuged at 1000 g for 15 minutes at 4°C. The SOD activity in the supernatant was measured using the SOD Assay Kit-WST (Sigma-Aldrich, USA) and standardized with SOD from bovine erythrocytes (Sigma Chemical Co., St. Louis, MO, USA). Nitrate (NO_3^-) + nitrite (NO_2^-) were measured in the supernatant using the Cayman Chemical Nitrate/Nitrite Colorimetric Assay kit (Cayman Chemical Co., Ann Arbor, MI, USA). This assay converts NO_3^- to NO_2^- with nitrate reductase, and total NO is measured as total nitrite using the Griess reagent.

Statistical analysis

Mann Whitney test was used for statistical evaluation of the results comparing laser treated and non-treated lesions. Results are expressed as mean values ± SEM, and p values lower than 0.05 were considered to be statistically significant.

RESULTS

Histopathological analysis

The paracoccidioidomycotic lesions obtained from non-treated animals had the same pattern at the 8, 9 and 10 days post infection, showing a disorganized epidermis and superficial dermis with an intensive mixed inflammatory infiltrate where some fungi can be seen (Figure 1A). At the center of the lesion, a substantial necrotic center with draining of fibrinous exudate, cellular debris and viable fungi was visible (Figure 1B). The animals that were treated with one session of laser irradiation (T1) showed the same morphological pattern observed with the untreated specimens, but with hyalinization of the collagen fibers on the connective tissue and lack of vacuolated macrophages against a larger area of granulomatous inflammation (data not shown). In mice treated with two sessions of laser irradiation (T2) the epidermis and superficial dermis showed greater histologic organization, milder mononuclear infiltrate and a light increase in collagen deposition (Figure 1C). At the center of the lesion, there was still a substantial necrotic center, but less viable fungi and a mixed inflammatory infiltrate are seen (Figure 1D). After three laser sessions (T3), the dermis presented a normal histological appearance with an intense collagen deposition (Figure 1E). At this time, the lesions also presented a higher organization of the granulation tissue pointing to the encapsulation of the lesion (Figure 1F).

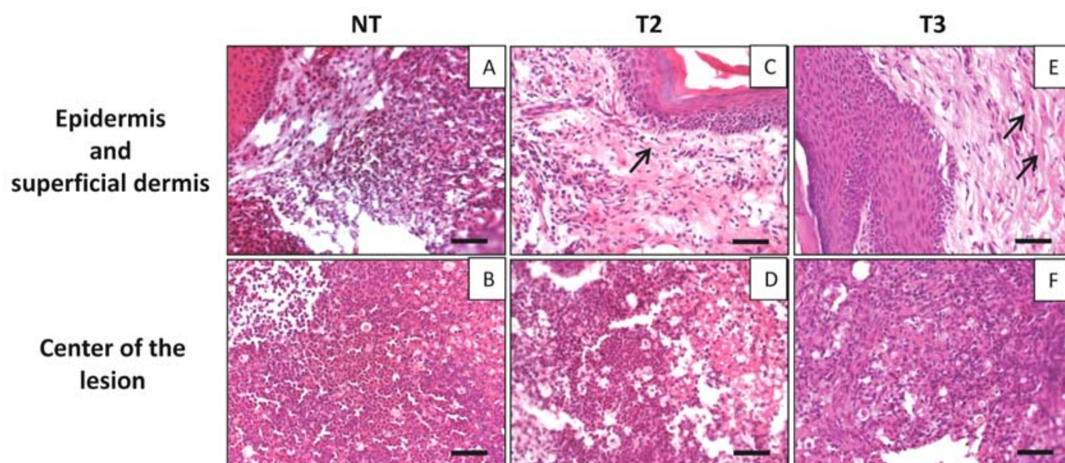

Figure 1. Histopathological features of PCM lesions treated or not with HeNe laser. Animals were inoculated with yeast forms of *P. brasiliensis* into the left back footpad, and 7, 8 and 9 days following infection the lesions were irradiated with laser. (A and B: non laser-treated animal (NT); C and D: animals treated with 2 laser sessions (T2); E and F: animals treated with 3 laser sessions (T3). Note that laser-treated lesions show greater histologic organization, milder mononuclear infiltrate and an increase in collagen deposition (arrows). The figures are representative of at least three experiments performed on different experimental days. Bar: 50 µm.

Flow cytometry

Cell populations present in the experimental PCM footpad lesions were analyzed by flow cytometry (Figure 2).

The analysis of lymphocyte subsets revealed a significant increase in the percentage of cells expressing NK and NKT cells associated markers soon after the first day of treatment (T1). After two (T2) and three laser sessions (T3), a gradual reduction of these subpopulations was observed in the lesions and the same cell percentage in laser treated and non-treated animals at days 9 and 10 post infection was found (Figure 2A/D).

The analysis of the total phagocytes population showed that these cells correspond to more than 80% of the total cells present in the lesion, and no significant differences between laser treated and non-treated groups could be demonstrated (Figure 2B/D).

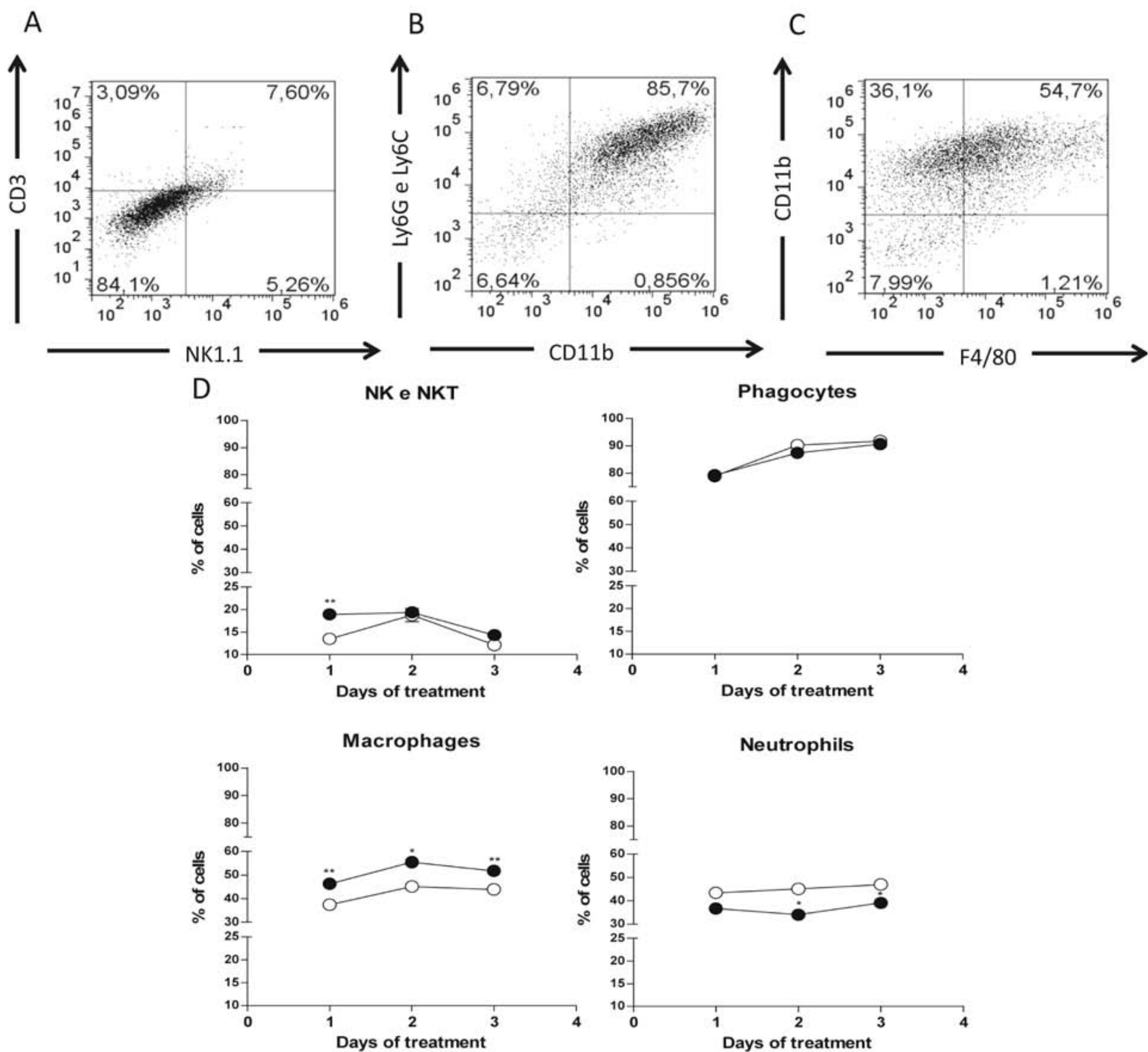

Figure 2. Analysis of cell populations at the experimental PCM lesion. A, B and C are representative dot-plots for: NK and NKT cell subsets, total phagocytes, and macrophage population, respectively. D: percentage of cells in lesions from non-treated (○) and laser treated animals (●). A higher percentage of macrophages and NK and NKT cells can be observed on the lesions after HeNelaser irradiation. Results are expressed as mean ± standard error (SEM) for at least five animals. * $p<0.05$; ** $p<0.01$.

However, the percentage of macrophages was higher (Figure 2C/D), while the percentage of neutrophils was significantly lower, in laser-treated animals than in the non-treated controls (Figure 2D).

Immunohistochemistry

The analysis of iNOS production, by the immunohistochemistry technique, revealed an increased expression in laser treated animals when compared to non-treated ones (Figures 3A to 3D). While non-treated animals showed weak labeling for iNOS (Figure 3A), laser treated animals presented strong labeling for this molecule after one (T1), two (T2) or three treatments (T3) (Figures 3B, 3C and 3D, respectively). These results were quantitatively confirmed by histometric computer-based analyses (Figure 3E).

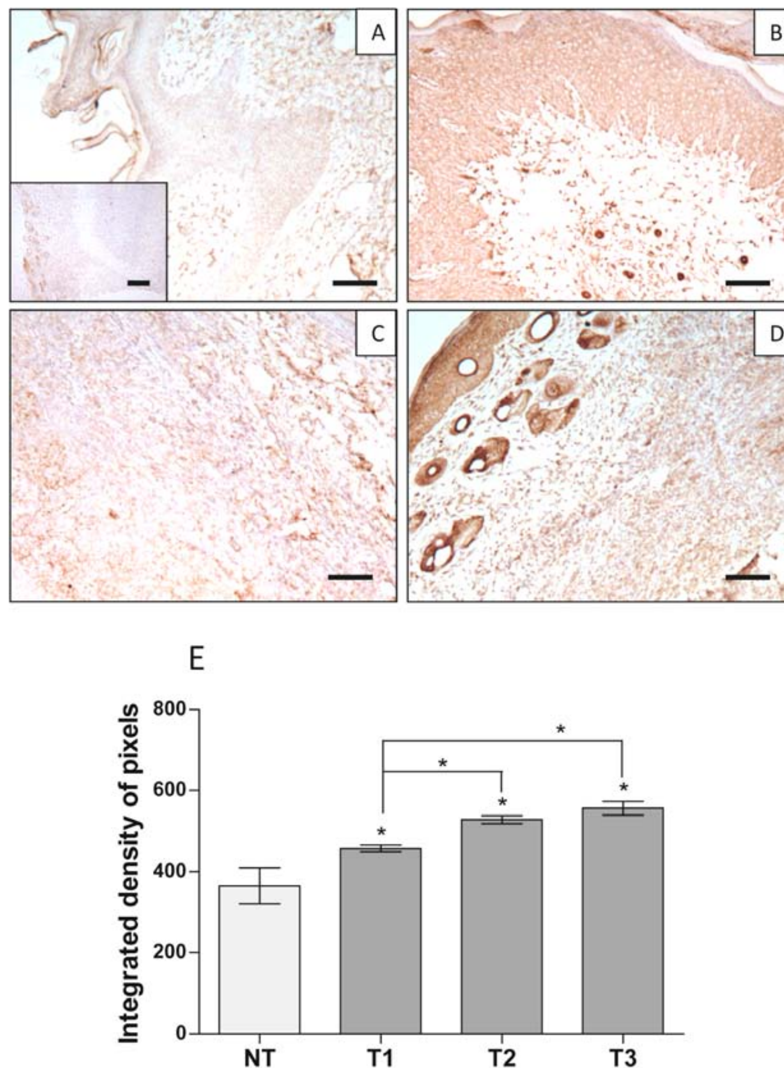

Figure 3. Immunohistochemistry staining for iNOS. A: Non-treated animals (NT); B to D: animals treated with one (T1), two (T2) or three (T3) laser irradiation session, respectively. Inset panel depicts negative control; no primary antibody. Note that laser-treated lesions present strong labeling for iNOS after treatment. The figures are representative of at least three experiments performed on different experimental days. Bars: 250 µm (inset) and 100 µm. E: The graphic corresponds to quantitative analysis of selected microscopic fields of lesions from laser-treated and non-treated lesions in terms of iNOS. Results are expressed in total pixels and represent the mean ± SEM for at least five animals. *p<0.05.

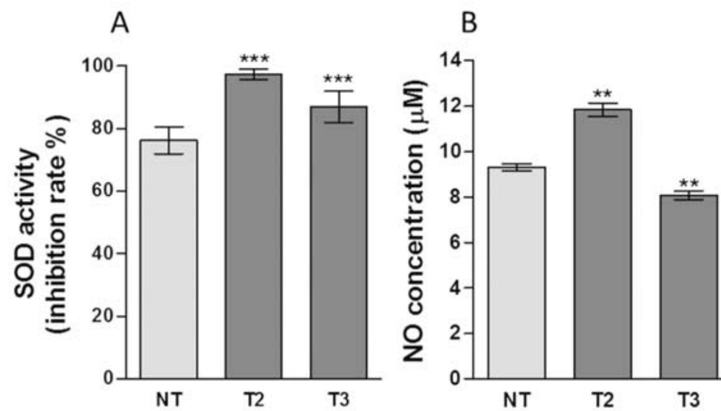

Figure 4. Superoxide dismutase (SOD) activity and tissue nitric oxide (NO) concentration in lesions from non-treated (NT) mice and after two (T2) or three (T3) laser treatment. Both the SOD activity and the NO concentration show a significant increase after two laser irradiation sessions and a reduction after three sessions of laser treatment. Results are expressed as mean ± standard error (SEM) for at least five animals. ** $p<0.01$; *** $p<0.0001$.

Superoxide dismutase (SOD) activity and nitric oxide (NO) concentration

The laser treatment of the PCM lesions leads to dramatic changes in the superoxide dismutase activity and nitric oxide production when compared to the non-treated group.

The SOD activity showed a significant increase after two laser irradiation sessions (T2). After three laser irradiation sessions (T3) the enzyme activity was reduced, but it remained significantly higher than the non-treated animals (Figure 4A). The nitric oxide concentration also significantly increased after two sessions of laser irradiation (T2). After the third day of treatment (T3), however, tissue NO concentration was diminished, remaining considerably lower than the non-treated group (Figure 4B).

DISCUSSION

The study of skin and soft-tissue lesions in PCM is important not only because of their frequency, difficulty of treatment and diagnostic value for the disease but also because they predispose patients to secondary bacterial infections [6, 7, 19].

Because of the discomfort evoked by PCM lesions and collateral drugs effects, the HeNe laser irradiation can represent an adjunctive, or even an alternative, therapy since its microbicidal effect, wound healing properties and non-invasive characteristics are already well known [9, 10, 20-23]. Although the exact mechanism of laser therapy remains obscure, it is known that it reduces the time and improves the quality and tensile strength of tissue repair, has anti-inflammatory effects, and provides reduction of painful symptoms at injured tissue areas [12, 13, 24, 25].

In leukocytes, the therapeutic effect of HeNe laser begins with photo-chemical reactions that are able to enhance the phagocytic activity and to increase the synthesis of bactericidal substances, like nitric oxide (NO) and peroxide anion, by a mechanism that involves the increased permeability of cell membranes to ions including Ca^{2+} [23-25]. Moreover, under the action of HeNe laser the enzyme superoxide dismutase (SOD), which is responsible for catalyzing the reaction that converts superoxide radicals into oxygen and hydrogen peroxide (a potent killer of microbes), is reactivated [24, 26].

Histopathological results presented here showed that laser treatment of lesions was efficient in accelerating tissue repair since epidermis and dermis are better organized with increased collagen deposition and granulation tissue formation, which represent typical processes of fibrogenesis. Evidence that laser photostimulation increases collagen synthesis has already been obtained from different studies [14, 27-29]. Beyond these effects, lesions that were exposed to three sessions of HeNe laser irradiation present less viable fungi and this fact certainly minimizes fungal infection dermal effects.

NK and NKT lymphocyte subpopulations are critical in protection against pathogens [30-32]. In experimental PCM, NK cells are very effective during the precocious phase of the disease and are involved in natural host resistance against the fungus since they would be potential sources of interferon-gamma (IFN-γ) and granulocyte-macrophage colony-stimulating factor (GM-CSF) at an early stage of infection [30-34]. Data presented here show an increase in the percentage of NK and NKT cells in laser-treated animals. Previously, we have demonstrated increased levels of IFN-γ in animals treated with the laser, which may be, at least partially, produced by NK cells [14]. The increase of these cells in animals treated with one session of irradiation may be the result of a major attraction of these cells to injury, helping to control and limit the spread of the pathogen by both directly and/or inducing death by producing IFN-γ that is important for phagocyte activation.

The prolonged presence of neutrophils in PCM lesions has long been reported [18, 35, 36]. However, neutrophils are less efficient in eliminating *Paracoccidioides brasiliensis* as compared with macrophages, since many of them are susceptible to infection due to deficiencies in the process of phagocytosis of pathogens [37, 38]. Here, we observed that laser-treated lesions show reduced percentage of neutrophils and substantially increased percentage of macrophages.

Macrophages play a central role in wound healing and are the main phagocytic cells in tissue repair, removing pathogens in addition to the remaining fragments (debris) from tissues, cells and matrix [39, 40]. When activated at the injury site, they secrete large amounts of growth factors and cytokines amplifying the initial signals released by degranulation of platelets and neutrophils [39]. Some growth factors such as fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) stimulate proliferation of endothelial cells, which are likely to have pro-angiogenic activity in tissue healing [39]. So, the greatest number of macrophages in laser-treated lesions can indicate an antifungal response more efficient as well as a healing process more advanced, which is consistent with the histopathological findings.

It is well established that activated macrophages kill phagocytosed pathogens by converting molecular oxygen into reactive oxygen intermediates (ROIs), and by producing reactive nitrogen intermediates, particularly nitric oxide (NO), through the action of an enzyme called inducible nitric oxide synthase (iNOS). Indeed, in the current experimental model of PCM, we have observed that laser-treated mice present significant increase in iNOS protein expression and NO release.

This increase in NO release observed in laser-treated animals, is certainly related to greater fungicidal activity of macrophages and increased resistance to infection PCM, as already observed by other authors [41, 42]. Our experimental model also revealed that after three laser sessions the NO released is reduced. This may be related to an accelerated healing because of a greater elimination of the pathogen, and, therefore, a milder inflammatory process. In fact, the literature has shown that an overproduction of NO, or a continuous production over a long period, can enhance host susceptibility to the fungus, making wound healing difficult [43, 44]. *In vitro* experiments to evaluate the formation of nitric oxide, peroxynitrite, and superoxide anion radical in mice peritoneal macrophages exposed to HeNe laser radiation are being conducted in our laboratory and such results certainly will improve our understanding regarding the effects of laser therapy on macrophages.

Data presented here also show significant differences between laser-treated and non-treated animals in relation to the superoxide dismutase (SOD) activity. SOD is an enzyme which catalyzes the dismutation of superoxide radicals ($O_{2^-} + O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$) and such a reaction may play an important role in PCM lesion for two reasons. The first reason is that high concentrations of superoxide radical (O_2-) can delay the healing process because of its interaction with NO to form peroxynitrite-anion with the subsequent formation of hydroxyl radical, which has an extremely potent cytotoxic effect [24, 45]. The second reason is that increased generation of hydrogen peroxide (H_2O_2) plays an important role in the elimination of microbes [42, 46, 47]. Thus, the increase in SOD activity observed in laser-treated animals results in both the reduced concentration of

superoxide, which leads to an acceleration of wound healing, and a greater release of H_2O_2, which is an important inflammatory mediator involved in the resistance against *P. brasiliensis* [48, 47, 49, 50].

We have also evaluated the direct action of HeNe laser on *Paracoccidioides brasiliensis* yeast cells. Our results show that HeNe laser is able to decrease viability and fungal growth, as well as to increase the expression of fungal structures that are used by the pathogen as virulence mechanisms (personal report). It is most probably that these effects also play an important role in accelerating the wound healing process.

CONCLUSION

Altogether our results indicate a dual effect of the laser treatment, decreasing the inflammatory response and accelerating the wound healing of the lesions. In this sense, we believe that the HeNe laser can be considered as an effective adjunctive treatment to be combined with conventional drug therapy for improving the treatment of painful non-healing paracoccidioidomycotic wounds.

ACKNOWLEDGEMENTS

We appreciate the valuable technical assistance with the animals provided by Marcos C. Meneghetti. We are indebted to Dr[a] Maria Alice da Cruz Höfling for her important collaboration with the microscopy and Dr[a] Elza Costa Cruz Vasconcellos for the laser apparatus. The authors are pleased to acknowledge financial support by the state-granting agency (FAPESP/ #2010/08485-5).

REFERENCES

1. Restrepo, A. 1985, Sabouraudia, 23, 323-334.
2. Severo, L. C., Geyer, G. R., Londero, A. T., Porto, N. S. and Rizzon, C. F. 1979, Mycopathologia, 67, 115-118.
3. Brummer, E., Castaneda, E. and Restrepo, A. 1993, Clin. Microbiol. Rev., 6, 89-117
4. Restrepo, A. 2000, Med. Mycol., 38, 317-322.
5. Borges-Walmsley, M. I., Chen, D., Shu, X. and Walmsley, A. R. 2002, Trends Microbiol., 10, 80-87.
6. Lupi, O., Tyring, S. K. and McGinnis, M. R. 2005, J. Am. Acad. Dermatol., 53, 931-951.
7. Trent, J. T. and Kirsner, R. S. 2003, Adv. Skin Wound Care, 16, 122-129.
8. Andriole, V. T. 1998, J. Antimicrobial Chemotherapy, 44, 151-162.
9. Jovanovic, L., Mirkovic, B. and Zivkovic, B. 1998, The Scientific Journal Facta Universitatis, 5, 61-63.
10. Yu, H., Chang, K. L., Yu, C. L., Chen, J. W. V. and Chen, G. S. 2003, J. Invest. Dermatol., 120, 56-64.
11. Qadri, T., Miranda, L., Tunér, J. and Gustafsson, A. 2005, J. Clin. Periodontol., 32, 714-719.
12. Karu, T. 1989, Health Phys., 56, 691-704.
13. Pugliese, L. S., Medrado, A. P., Reis, S. R. and Andrade, Z. A. 2003, Pesquisa Odontol. Bras., 17, 307-313.
14. Ferreira, M. C., Brito, V. N., Gameiro, J., Costa, M. R., Vasconcellos, E. C., Cruz-Hofling, M. A. and Verinaud, L. 2006, J. Photochem. Photobiol. B, Biol., 84, 141-149.
15. Ferreira, M. C., Gameiro, J., Nagib, P. R. A., Brito, V. N., Vasconcellos, E. C. and Verinaud, L. 2009, Photochem. Photobiol., 85, 227-233.
16. Nagib, P. R. N., Gameiro, J., Alves da Costa, T., Di Gangi, R., Ribeiro, J. S., Paulino, L. C. and Verinaud, L. 2010, Photochem. Photobiol., 86, 920-924.
17. Sano, A., Nobuyuki, K. and Iabuki, K. 1993, Mycopathologia, 124, 157-161.
18. Collins, T. J. 2007, BioTechniques, 43, 25-30.
19. Franco, M. F. and Montenegro, M. R. G. 1982, Anatomia Patológica. In G., Del Negro, C. S. Lacaz, and A. M. Fiorillo, (Eds.) Paracoccidioidomicose - blastomicosesul-americana. Sarvier- EDUSP, 97-117.
20. Mester, E., Spiry, T., Szende, B. and Tota, J. G. 1971, Am. J. Surg., 122, 532-535.
21. Vladimirov, Y. A., Osipov, A. N. and Klebanov, G. I. 2004, Biochem., 69, 81-90.
22. Gigo-Benato, D., Geuna, S. and Rochkind , S. 2005, Muscle Nerve, 31,694-701.
23. Posten, W., Wrone, D. A., Dover, J. S., Arndt, K. A., Silapunt, S. and Alam, M. 2005, Dermatol. Surg., 31, 334-40.

24. Klebanov, G. I. and Poltanov, E. A. 2003, Laser Physics, 13, 70-83.
25. Chung, H., Dai, T., Sharma, S. K., Huang, Y. Y., Carroll, J. D. and Hamblin, M. R. 2012, Ann. Biomed. Eng., 40, 516-33.
26. Klebanov, G. I., Poltanov, E. A., Chichuk, T. V., Osipov, A. N. and Vladimirov, Y. A. 2005, Biochem., 70, 1335-40.
27. Saperia, D., Glassberg, E., Lyons, R. F., Abergel, R. P., Baneux, P., Castel, J. C., Dwyer, R. M. and Uitto, J. 1986, Biochem.Biophys. Res. Commun., 138, 1123-1128.
28. Reddy, G. K., Stehno-Bittel, L. and Enwemeka, C. S. 1998, Lasers Surg. Med., 22, 281-287.
29 Lubart, R., Eichler, M., Lavi, R., Friedman, H. and Shainberg, A. 2005, Photomed. Laser Surg., 23, 3-9.
30. Peraçoli, M. T., Soares, A. M., Mendes, R. P., Marques, S. A., Pereira, P. C. and Rezkallah-Iwasso, M. T. 1991, J. Med. Vet. Mycol., 29, 373-80.
31. Peraçoli, M. T., Fortes, M. R., Da Silva, M. F. and Montenegro, M. R. 1995, Rev. Inst. Med. Trop. São. Paulo, 37, 129-36.
32. Silveira, P. C., Silva, L. A., Freitas, T. P., Latini, A. and Pinho, R. A. 2011, Lasers Med. Sci., 26, 125-31.
33. Dunn, P. L. and North, R. J. 1991, Listeriosis. Infect. Immun., 59, 2892-900.
34. Pagliari, C., Pereira, N. V., Kanashiro, L., Stegun, F. W., Croda, J., Duarte, M. I. and Sotto, M. N. 2010, J. Cutan. Pathol., 37, 565-570.
35. Calich, V. L., Singer-Vermes, L. M., Siqueira, A. M. and Burger, E. 1985, J. Exp. Pathol., 66, 585-94.
36. Levitz, S. M. and North, E. A. 1996, Infect. Immun., 64, 1595-9.
37. Goihman-Yahr, M., Essenfeld-Yahr, E., Albornoz, M. C., Yarzábal, L., Gómez, M. H., San Martín, B., Ocanto, A., Gil, F. and Convit, J. 1980, Infect. Immun., 28, 557-66.
38. Acorci, M. J., Dias-Melicio, L. A., Golim, M. A., Bordon-Graciani, A. P., Peraçoli, M. T. and Soares, A. M. 2009, J. Immunol., 69, 73-9.
39. Leibovich, S. J. and Ross, R. 1975, Am. J. Pathol., 78, 71-100.
40 Dias, M. F., Mesquita, J., Filgueira, A. L. and De Souza, W. 2008, Med. Mycol., 46(3 Suppl.), 241-9.
41. Martin, P. 1997, Science, 276, 75-81.
42. Gonzalez, A., de Gregori, W., Velez, D., Restrepo, A. and Cano, L. E. 2000, Infect. Immun., 68, 2546-52.
43. Nascimento, F. R., Calich, V. L., Rodríguez, D. and Russo, M. 2002, J. Immunol., 168, 4593-600.
44. Moreira, A. P., Dias-Melicio, L. A., Peraçoli, M. T., Calvi, S. A. and Soares, A. M. 2008, Mycopathologia, 166, 17-23.
45. Nishikaku, A. S., Molina, R. F., Ribeiro, L. C., Scavone, R., Albe, B. P., Cunha, C. S. and Burger, E. 2009, Med. Microbiol. Immunol., 198, 123-35.
46. Ducrocq, C., Blanchard, B., Pignatelli, B. and Ohshima, H. 1999, Cell Mol. Life Sci., 55, 1068-77.
47. Carmo, J. P., Dias-Melicio, L. A., Calvi, S. A., Peraçoli, M. T. and Soares, A. M. 2006, Med. Myco., 44, 363-8.
48. Phan, T. T., Wang, L., See, P., Grayer, R. J., Chan, S. Y. and Lee, S. T. 2001, Biol. Pharm. Bull., 24, 1373-9.
49. Rdrigues, D. R., Dias-Melicio, L. A., Calvi, S. A., Peraçoli, M. T. and Soares, A. M. 2007, Med. Mycol., 45, 27-33.
50. Kerkweg, U., Petrat, F. and Korth, H. G. 2007, Shock, 27, 552-558.