Low-level laser therapy and micro current in burn wound healing in rats. Associated or isolated therapy?

Laserterapia e microcorrente na cicatrização de queimadura em ratos. Terapias associadas ou isoladas?

Laserterapia y microcorriente en la cicatrización de quemaduras en ratas. ¿Terapias asociadas o aisladas?

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ABSTRACT | This study aimed to investigate if there are differences between the associated and isolated therapies from the laser and micro current on healing of burn wound healing in rats. A total of 40 male rats were randomly allocated into four groups: control group (CG), micro current group (MG), laser group (LG) and laser/micro current group (LMG), treated with associated laser and micro current. Thermal damage was done on the back of the animal and a total of ten days therapy was performed. After treatment samples were taken from the lesions to perform semi quantitative histopathological study using Hematoxylin Eosin and Masson Trichrome. The Kruskal-Wallis and Dunn's Test were used for statistical analyses. We observed a significant difference between groups for production of fibroblasts (p=0.0003), collagen (p=0.0153), neoangiogenesis (p=0.0031) and skin annexes (p=0.0004). In semi-quantitative histological analysis, the LMG showed lower values in presence of collagen, fibroblasts and number of skin appendages, only for neoangiogenesis, the associated therapy showed similar values to single modality therapy groups. Laser and microcurrent have beneficial effects on tissue healing. However, it is suggested that the association of these two therapies reduces the effectiveness of the treatment when compared to single mode treatment.

Keywords | combined modality therapy; lasers; electric stimulation; wound healing.

RESUMO | Este estudo teve o objetivo de investigar se há diferenças entre as terapias associadas e isoladas do *laser* e microcorrentes no reparo de lesão por queimadura em ratos. Um total de 40 animais foi dividido aleatoriamente em quatro grupos: grupo controle (GC); grupo microcorrente (GM), grupo *laser* (GL) e grupo *laser*/microcorrente (GLM), tratados com *laser* associado a microcorrentes. Após lesões térmicas induzidas no dorso do animal, foi realizado um total de dez dias de tratamento. Amostras do tecido foram coletadas para estudo histopatológico semiquantitativo com Hematoxilina Eosina e Tricrômico de Masson. Foram utilizados os testes de Kruskal-Wallis e *post-hoc* de Dunn. Houve diferença significativa entre os grupos para a produção de fibroblastos (p=0,0003), colágeno (p=0,0153), neoangiogênese (p=0,0031) e anexos cutâneos (p=0,0004). Na análise histológica semiquantitativa, o GLM apresentou valores menores nos parâmetros histológicos de presença de colágeno, número de fibroblastos e anexos cutâneos (p<0,05) em relação às terapias isoladas, exceto para a neoangiogênese, cujos valores da terapia associada foram semelhantes aos grupos de terapia com modalidade única. Apesar do *laser* e da microcorrente separadamente terem efeitos benéficos para a cicatrização tecidual, a associação das modalidades parece ter diminuído a ação de reparo. No entanto, sugere-se que a associação destes

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recursos parece diminuir os efeitos do tratamento quando se comparam os grupos de modalidade única.

Descritores | terapia combinada; lasers; estimulação elétrica; cicatrização.

RESUMEN | Este estudio tiene el objetivo de investigar si hay diferencias entre las terapias asociadas y aisladas del *láser* y microcorrientes en la reparación de lesión por quemadura en ratas. Un total de 40 animales fueron divididos aleatoriamente en cuatro grupos: grupo control (GC), grupo microcorriente (GM), grupo *láser* (GL) y grupo *láser*/microcorriente (GLM), tratados con *láser* asociado a microcorrientes. Después de inducidas las lesiones térmicas en el dorso del animal, fueron realizados en total diez días de tratamiento. Las muestras de tejido fueron recolectadas para el estudio histopatológico semicuantitativo usando Hematoxilina Eosina y Tricómico de Masson. Fueron utilizados los tests de Kruskal-Wallis y *post-hoc* de Dunn's. Hubo diferencia significativa entre los grupos para la producción de fibroblastos (p=0,0003), colágeno (p=0,0153), neoangiogénesis (p=0,0031) y anexos cutáneos (p=0,0004). En el análisis histológico semicuantitativo, el GLM presentó valores menores en los parámetros histológicos de presencia de colágeno, número de fibroblastos y anexos cutáneos (p<0,05) en relación a las terapias aisladas, excepto para la neoangiogénesis, cuyos valores de la terapia asociada fueron semejantes a los grupos de terapia con modalidad única. A pesar de que el láser y la microcorriente de forma aislada tienen efectos benéficos para la cicatrización del tejido, la asociación de las modalidades parece haber disminuido la acción de la reparación. El láser y las microcorrientes son efectivos en acelerar el proceso de reparación del tejido. Sin embargo, se sugiere que la asociación de estos recursos parece disminuir los efectos del tratamiento cuando son comparados con los grupos de modalidad única.

Palabras clave | terapia combinada; Terapia *laser* de baja intensidad; *lasers*; estimulación eléctrica; cicatrización de heridas.

INTRODUCTION

In physiotherapy, several resources are being used to accelerate and improve the quality of the regenerative process, such as lasers, microcurrents, ultrasounds, and ultraviolet radiation[1-3]. These resources accelerate the healing process, acting upon the sequence of physiological and biochemical events of this process through inflammation, collagen synthesis, formation of granulation tissue, and re-epithelization[1,2].

Through the analysis of cellular structures that are activated and/or inhibited by these resources, studies[2,4,5] performed on laboratory animals have contributed to the elucidation of which physiotherapy resources might promote a regenerative process of quality. The therapeutic effects have been attributed to the interaction between external energetic stimuli and the biological tissue (biostimulation), promoting an increase in cellular activities during the healing process.[5,6]

Among the modalities used, the low-intensity laser therapy is highlighted[6-8]; it shortens the time for remodeling, and improves the quality of the tissue in neoformation[1,6]. The basic biological mechanism promoted by this eletrophysical resource seems to be the absorption of red and infrared light by chromophores contained in the protein components of the respiratory chain located in the mitochondria, which, in turn, initiate a torrent of biochemical events upon absorbing energy, and this results in an increase of enzymatic activity, production of triphosphate adenosine, protein synthesis, cellular proliferation, deposition, and collagen organization[9-10].

Other authors[11-12] suggest that the electric stimulation by microcurrents also accelerates ATP synthesis, has antioxidant effect, stimulates transmembrane transport, and reestablishes tissue bioelectricity, promoting reduction of the inflammatory process, pain relief, and healing acceleration. In this way, the modality would aim at normalizing the flow of the currents, which may be interrupted when tissue lesion occurs due to burning[13-14].

Thus, laser and microcurrent therapies have positive effects on the acceleration of the healing process when used separately. In physiotherapists' clinical practice, the associated use of these therapies as a way of potentiating the effects mentioned above has been observed. However, few studies describe this associated effect, and, in this way, the real results have yet to be discussed. Studies[7,15] describe that when multi-therapy is used in healing processes, the beneficial effects might neutralize each other. Among the several experimental models, our study used one of thermic lesion in rats because of the possibility of easily applying the therapies, and of assessing all the necessary histological parameters for the comparison of the treatments.

Therefore, the aim of the present study was to investigate if there are differences between associated and isolated laser and microcurrent therapies in tissue healing in a model of burn wounds in rats.

MATERIAL AND METHODS

Forty Wistar rats (*Rattus norvegicus*) were used, weighing between 250 and 300 grams, chosen randomly, provided by the UNP bioterium. Random allocation into four groups was performed (n=10): microcurrent group (MG), laser group (LG), control group (CG), and laser/microcurrent group (LMG). All rats were submitted to the same environment and biological day/night cycle with 10 to 12 hours of controlled exposure to light, temperature and illumination, humidity maintained by air conditioning, and minimum noise. The animals remained in individual polypropylene cages lined with laboratory-grade pine shavings as bedding, and received Labina® chow and water. The therapies were performed in the afternoon (2 to 5 pm). For the four groups, the procedures were performed in the following sequence: (a) administering of dissociative anesthetic Zoletil® with dosage of 50 mg/kg intramuscularly in the quadriceps muscle; (b) trichotomy on the animal's back; (c) induction of a second-degree burn wound[2] with a 6x3 cm aluminum plate pressured against the animal's back 4 cm away from the skull base during 10 seconds; the plate was previously warmed in becker with water at 100°C for a period of 10 minutes, as used by Meyerholtz et al.[2] and Meireles et al.[16] (Figure 1). The proposed treatments were initiated immediately after the lesions, and performed daily during 10 days. The CG was submitted to the same experimental protocol, with exception of the exposition to the treatments.

For the application of laser, the device Photon Laser III of the brand DMC® of São Carlos, SP, was utilized; it carries the following features: visible laser (AlGaInP) in the 660 nm range, continuous mode, 30 mW power, 10 J/cm^2 dosage, and energy of 0.3 J per application spot during 9 seconds per spot inside the burn wound. In the region adjacent to the wound (borders) the same power was used but with dosage of 12 J/cm^2 and energy of 0.33 J per spot during 11 seconds. The application was performed through spot technique in direct contact with the wound, where the probe was positioned with light pressure at an angle of 90 degrees. The interval of 1.5 cm between the spots was respected, totalizing 6 spots on the burn wound and 14 spots in its adjacent region, totalizing 20 spots per animal. For the microcurrent, the device Physiotonus Microcurrent Stimulator of the brand Bioset® was used through the application of a square monophasic pulsated current of reversible polarity each 2.5 seconds, with an intensity of 160 μA and frequency of 60 Hz during 15 minutes through two adhesive silicon electrodes (Valutrode®) with 3.2 cm^2 of diameter positioned at the extremities of the wound.

For the MLG group, laser therapy was performed first, and then the microcurrent treatment. The two instruments were previously calibrated and warranted by their suppliers.

This study was approved by the Research Ethics Committee of the Potiguar University (UNP), file number 062/2008.

Histology

After 24 hours from the last therapy application, the animals were sacrificed in a closed chamber with liberation of CO_2. In the instant following the sacrifice, the biopsy of the skin tissue was performed 7 cm away from the skull base for the purposes of a histological study, including the wound in its healing process, the wound border, and part of the skin adjacent to the wound border. The samples were fixated in formalin, inserted in paraffin blocks, taken to the microtome, and cut in sections of 5 μm of thickness. The sagittal sections were kept in a drying chamber, and the cuts were posteriorly submitted to coloration by hematoxylin-eosin and Masson's trichrome. The tissue analysis was performed by a blind evaluator with the use of a Nikon® (Nikon, Tokyo, Japan) optical microscope. All the criteria applied in the semi-quantitative histological analysis[16-17] were verified through scores in a scale from 0 to 3 (Table 1).

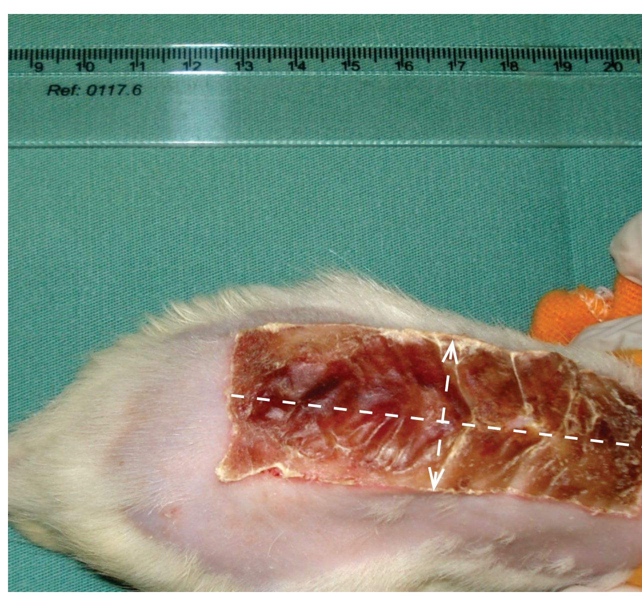


Figure 1. Burn model used. Adapted from Meireles et al.[16] Wound in the healing process, with loss of epidermis and no sign of infection. The biopsy of the animal's skin was extracted from the middle point between the dotted lines (in white) in the figure

Table 1. Criteria for histological analysis

Histology	Score			
	Null=0	Light=1	Moderate=2	Intense=3
Re-epithelization	Absent	Present: comprising <50% of the wound	Present: comprising >50% of the wound	Present: covering 100% with regular thickness
Acute inflammation	Absent	<25% of neutrophils	<25-50% of neutrophils	>50% of neutrophils
Number of fibroblasts	Absent	<25% fibroblasts younger and less differentiated among the other types of cell	<25-50% fibroblasts younger and less differentiated among the other types of cell	>50% fibroblasts younger and less differentiated among the other types of cell
Neoangiogenesis	Absent	Less than what was observed on the healthy adjacent tissue	Similar amount to the one observed on the healthy adjacent tissue	More than what was observed on the healthy adjacent tissue
Skin annexes	Absent	<25% in relation to what was observed on the healthy adjacent tissue	>25-50% in relation to what was observed on the healthy adjacent tissue	>50% in relation to what was observed on the healthy adjacent tissue
Number of collagen fibers	Absent	Red coloration less intense than that observed on the healthy adjacent tissue	Red coloration similar to that observed on the healthy adjacent tissue	Red coloration more intense than that observed on the healthy adjacent tissue

Adapted from Meireles et al.[16] and Iordanou et al.[17].

Statistical analysis

For the data analysis the software IBM SPSS® 19 and the GraphPad Prism® 5 were used. For the comparison of the intergroup non-parametrical averages, the Kruskal Wallis and the Dunn's post-test were used. We considered p<0.05 significant.

RESULTS

On the tenth day after the burn wound was inflicted, the appearance of the skin lesion in the LG, MG, and MLG pointed to loss of epidermis and hypodermis, representing a second-degree burn[2], with moderate acute inflammatory reaction but with a clean and uninfected wound. There was no significant difference among the groups in relation to epithelial regeneration (p=0.0568), and inflammatory process (p=0.9640). We observed a significant difference among the groups in the number of fibroblasts (p=0.0003), collagen (p=0.0153), neoangiogenesis (p=0.0031), and skin annexes (p=0.0004).

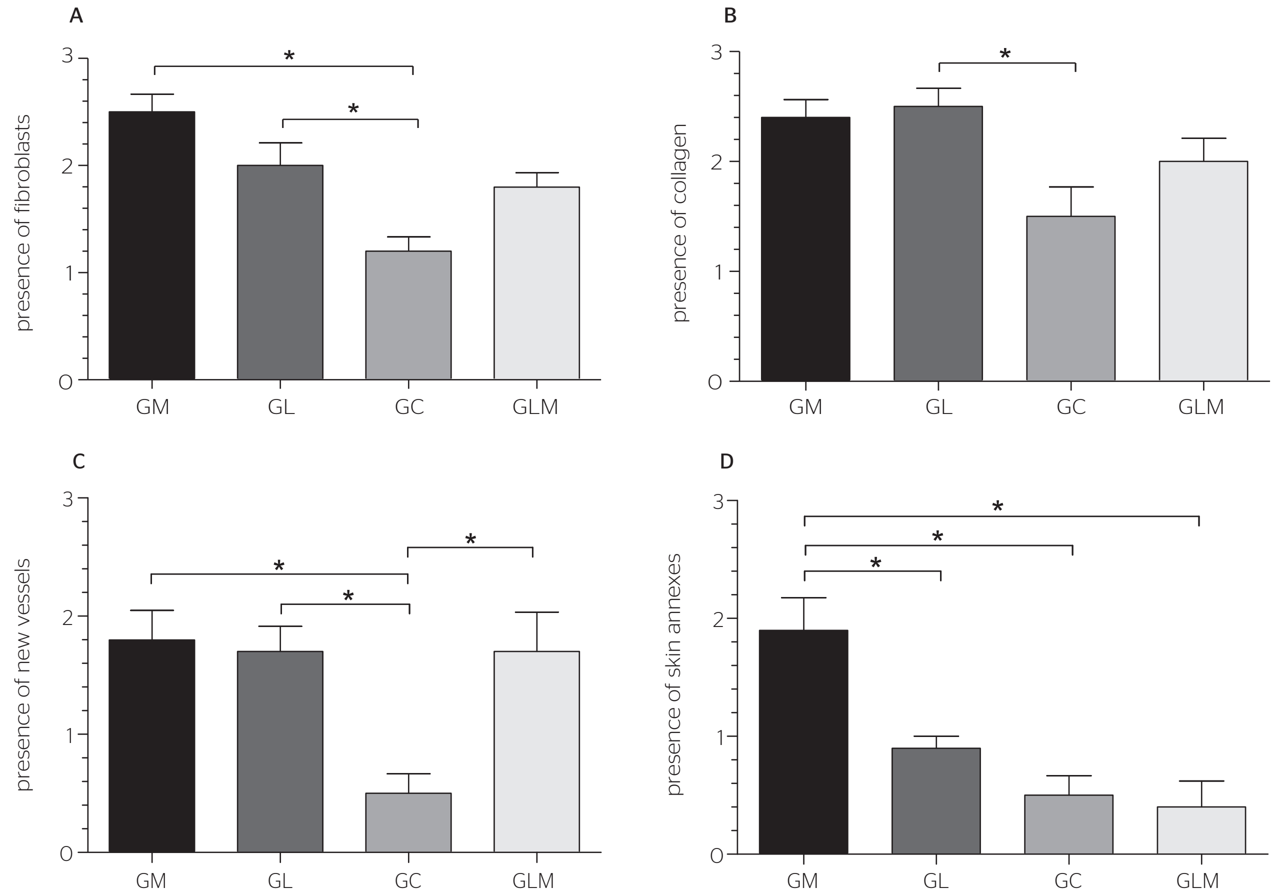
For an increase in the presence of fibroblasts, the application of only one of the modalities was more efficient than associated therapy (Figure 2A). The MG did not present any difference to the MLG in relation to the presence of collagen fibers (Figure 2B). There was a significant increase in neoangiogenesis in all groups treated in comparison to CG (Figure 2C); however, the associated therapy (MLG) did not present a significant difference when compared to the therapies applied separately (LG and MG). The MG registered significant improvement in comparison to others groups in relation to the presence of skin annexes (Figure 2D). The associated therapy had similar values to the groups of single-modality therapy only in relation to neoangiogenesis.

DISCUSSION

Through the use of a burn model on Wistar rats, it was observed that, when applied in association, the visible laser AlGaInp (660 nm and 30 mW power) and the microcurrent (160 μA and 60 Hz frequency) promoted significant improvement only in the formation of new blood vessels in comparison to the single-modality therapy. In all the other parameters evaluated, the individual use of one of the isolated therapies was better (fibroblasts, collagen, and skin annexes) than the joint therapy.

Evidence suggests that the use of red or infrared wave lengths in a series of dosage parameters (median of 4.2 J/cm^2), including the ones used in the present study, results in significant benefits on the healing of wounds in animal models and pathological processes in humans[18,19]. The use of laser in different wave lengths is capable of accelerating epidermal formation, increasing the thickness of epidermal layers, and promoting neovascularization and reorganization of collagen fibers[1,2,20-25]. The result of the treatment varies according to the parameters; the visible laser is utilized more often for being more superficial and for interacting specifically with superficial chromophores, adapting itself to the treatment of epithelial lesions[18,19,23].

Microcurrent therapy is also an efficient resource in the healing process[13-15]. In their study of the healing process in guinea pigs with the use of an electric



*Significant when p<0.05. MG: Microcurrent group; LG: laser group; CG: control group; MCG: laser/microcurrent group.
Figure 2. Intergroup comparison of the histological variants through Dunn's post-test

current of 50 μA, Agne et al.[24] described an increase in fibroblasts and inflammatory cells migration, and a greater alignment of collagen fibers, which contributed to healing. Using a microcurrent of 50 μA in the treatment of burn wounds in rats, Santos et al.[5] observed a number of fibroblasts and collagen superior to that of the control group. With microcurrents of 300 μA for 30 minutes/day, Demir et al.[15], observed improvement in cell proliferation and maturation, which stimulated fibroblast growth. These discoveries, positive in relation to the number of fibroblasts and the increase in the amount of collagen fibers, were also verified in our research. In vitro studies suggest that a microcurrent of 100 μA and laser promote the migration[26] and proliferation[23] of human dermal fibroblasts.

Laser and microcurrent represent an excellent therapy target in the promotion of neoangiogenesis during the healing process. The endothelial cells of the micro vessels seem to be sensitive to laser stimulation through the expression of gene proteins that regulate the cell cycle and the proliferation of these cells[27]. Bai et al.[28] describe that electric fields of 150 to 400 mV/mm also perform migration, reorientation, and extension of the endothelial cells of micro-circulation vessels.

The endothelial cells of micro-circulation present different behaviors when compared to macrovascular tissues, which suggests that each cellular type has a distinct disposition of receptors, and tolerance to different electric fields, contributing or not contributing to the activation of the vascular endothelial growth factor[28].

Even with the positive action of laser and microcurrent upon the healing process in the various model types, such as skin[1], diabetic ulcers[6], and temperature burns[5,16,24], their associated use still deserves more discussion.

Gum et al.[7] report the idea that the combined therapy might prompt an overdose of stimuli upon the cells, which leads to the annulment of therapeutic effects. In their study with laser and microcurrent, an improvement in the strength, elasticity, tension, and maximum effort in rabbit tendons was observed. The improvements brought by multi-therapy were consistent but less noticeable when compared to protocols of single modality. It is possible that the electric stimulation might have hampered the occurrence of cellular and molecular reactions involved in the healing process, such as the gene expression of cellular growth factors, errors in the process of cell differentiation, and alteration in the behavior of receptors and ionic channels. Considering that these act upon the cellular metabolism, we point to the hypothesis of cellular fatigue, and alterations in cellular signalization or in the metabolic ways of the cells[27,28].

Laser and microcurrent seem to act directly upon the expression of cellular growth factors in several types of cell (fibroblast, vascular endothelium, epithelial cells) related to the healing process. However, each type seems to possess a certain threshold (necessary dosage for positive effects) and tolerance (maximum dose to produce positive effects) to energetic stimuli.

Although laser and microcurrent are beneficial to tissue healing when used separately, their combination seems to decrease therapeutic action. The results recommend attention during the treatment of dermal burn lesions, and the suggestion of a therapy with these modalities used independently might be the best course of action. The biophysical and cellular action mechanisms that involve the combined use of therapeutic resources deserve broader investigation in order to obtain a more complete explanation of the phenomena analyzed.

CONCLUSION

This study concludes that, when applied separately, laser and microcurrent accelerate the healing process of burn wounds. However, when associated, they promote an improvement in neoangiogenesis only, and do not present significant improvement of the epithelial regeneration, the inflammatory process, collagen, fibroblasts, and skin annexes. We suggest that the association of both resources decreases the effects of treatment when compared to the single-modality groups.

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