Evaluation of Thermal Variation of Skin Wounds In Rats Subjected To Biomodulatory Therapies

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SUMMARY

INTRODUCTION: Biomodulatory therapies able to help tissue repair such as laser photobiomodulation and ozone therapy, have aroused the interest of many scientific communities. Infrared thermography is a diagnostic tool adept for verifying dysfunctions resulting from the healing process, especially when it is undergone to the influence of different therapeutic resources.

AIMS: To evaluate the effects of laser photobiomodulation, ozone gas and ozonized oil on the thermal variation of tissue in skin wounds in rats through thermographic analysis.

METHODS: This was an in vivo experimental study in which 40 male Wistar rats were submitted to cutaneous wounds to create standardized dorsal wounds using a circular scalpel of 6 mm diameter. The specimens were allocated in 4 groups containing 10 animals each. Three groups received treatment with laser photobiomodulation, ozone gas and ozonated oil, respectively. And were compared to the Control Group through thermograms of the wound area on the 5th and 10th post-operative day. The ANOVA test was used and the significance level was $p<0.05$.

RESULTS: A reduction in the average temperature in the wound area was observed in all experimental groups with greater variation of thermal coefficient in the treated groups ($p<0.05$). On the 5th day, Gas ozone and ozonated groups presented lower main temperature in comparison with control group and laser group (33.9°C±0.7, 34.7°C±0.4, respectively), as well as with thermal variation coefficient (ΔT= -1.8 and 1, respectively). On the 10th day, the thermographic averages of the basal temperature became closer, exception of the ozone gas group which maintained the average temperature higher at 35.1°C±0.4 ($p<0.05$) and higher ΔT (+1.8).

CONCLUSION: Infrared thermography proved to be effective for monitoring the thermal variation resulting from the inflammatory process, during cutaneous tissue repair undergone to the biomodulatory therapies using. Overall, with thermal imaging, ozone gas stood out from the other groups since it showed lower mean average temperatures during the inflammatory phase of the repair and also for raising the average temperature on the 10th study day for the remodeling phase.

KEYWORDS: Healing; ozone; Laser; Low power laser therapy; Infrared thermography.

BEWERTUNG DER THERMISCHEN VARIATION VON HAUTWUNDEN BEI RATTEN, DIE BIOMODULATORISCHEN THERAPIEN UNTERZOGEN WURDEN

EINLEITUNG: Biomodulatorische Therapien, die in der Lage sind, die Gewebereparatur zu unterstützen, wie z. B. die Laser-Photobiomodulation und die Ozontherapie, haben das Interesse vieler wissenschaftlicher Gemeinschaften geweckt. Die Infrarot-Thermografie ist ein diagnostisches Instrument, das geeignet ist, Funktionsstörungen zu überprüfen, die sich aus dem Heilungsprozess ergeben, insbesondere wenn dieser unter dem Einfluss verschiedener therapeutischer Maßnahmen erfolgt

ZIELE: Es sollten die Auswirkungen von Laser-Photobiomodulation, Ozongas und ozonisiertem Öl auf die thermische Variation von Gewebe in Hautwunden bei Ratten durch thermografische Analyse untersucht werden.

METHODEN: Hierbei handelte es sich um eine experimentelle In-vivo-Studie, in der 40 männliche Wistar-Ratten mit einem kreisförmigen Skalpell von 6 mm Durchmesser Hautwunden unterzogen wurden, um standardisiert Wunden am Rücken zu erzeugen. Die Proben wurden in 4 Gruppen mit je 10 Tieren eingeteilt. Drei Gruppen erhielten eine Behandlung mit Laser-Photobiomodulation, Ozongas bzw. ozonisiertem Öl. Diese wurden mit der Kontrollgruppe durch Thermogramme des Wundbereichs am 5. und 10. postoperativen Tag verglichen. Der ANOVA-Test wurde verwendet und das Signifikanzniveau betrug p<0,05

ERGEBNISSE: In allen Versuchsgruppen wurde eine Reduktion der mittleren Temperatur im Wundbereich beobachtet, wobei der thermische Koeffizient in den behandelten Gruppen stärker variierte (p<0,05). Am 5. Tag wiesen die Ozongas- und ozonisierten Gruppen im Vergleich zur Kontrollgruppe und der Lasergruppe (33,9 °C±0,7, 34,7 °C±0,4) sowie zum thermischen Variationskoeffizienten (ΔT= -1,8,bzw.1) eine niedrigere Haupttemperatur auf. Am 10. Tag näherten sich die thermographischen Mittelwerte der Basaltemperatur an, mit Ausnahme der Ozongasgruppe, welche die Durchschnittstemperatur mit 35,1 °C±0,4 (p<0,05) und einem höheren ΔT (+1,8) höher hielt.

SCHLUSSFOLGERUNG: Die Infrarot-Thermografie erwies sich als wirksam zur Überwachung der thermischen Schwankungen, die sich aus dem Entzündungsprozess während der Hautgewebereparatur während der biomodulatorischen Therapien ergeben. Insgesamt hob sich Ozongas mit Wärmebildaufnahmen von den anderen Gruppen ab, da es niedrigere mittlere Durchschnittstemperaturen während der Entzündungsphase der Reparatur und auch eine Erhöhung der Durchschnittstemperatur am 10. Untersuchungstag für die Umbauphase zeigte.

SCHLÜSSELWÖRTER: Heilung; Ozon; Laser; Lasertherapie mit geringer Leistung; Infrarot-Thermografie.

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Introduction

Biomodulatory therapies represented by laser photobiomodulation and ozone therapy have increasingly been investigated looking for obtaining standardized clinical protocols. Such therapies are able to induce analgesic, anti-inflammatory and tissue biomodulator effects that help to improve the healing pattern of different types of wounds [1-3].

With a wavelength that lies in the visible and invisible light spectrum, laser photobiomodulation positively impact on healing process through increased adenosine triphosphate (ATP) biosynthesis, collagen biosynthesis, lymphocyte proliferation and functional activity and number of fibroblasts [4,5]. Laser photobiomodulation may be characterized as triggering or inhibiting physiological, biochemical and metabolic processes through its photophysical or photochemical effects [6]. Such effects are interdependent and may vary according to the type of treated tissue, the fluence and irradiance of laser, as well as the time and intervals of application designed for the patient [6,7].

Ozone therapy has a therapeutic action based on activating redox mechanisms, protein synthesis and increasing the number of ribosomes and mitochondria in cells [8,9]. Thus, changes at the cellular level explain the increase in functional activity and regenerative potential of tissues and organs submitted to ozone treatment [10,11].

During the wound healing process, the microcirculation in the wound bed undergoes a complex change. In the first days, there is a predominance of the acute inflammatory phase which results in important hemodynamic shift with intense exudation and subsequent vascular proliferation [12,13]. Currently, an image resource has been used to assess the thermal coefficient variation resulting from the increase of circulatory pattern in a given tissue - infrared thermography [14]. This diagnostic tool captures the image with a special camera that maps the patient's body and transforms the detection of infrared emission from each anatomical site into temperature [15]. It was introduced as a diagnostic method in Medicine since 1960 [16]. However, with the advancement of technology in data collection, transfer and temperature measurement, it has significantly evolved in terms of sensitivity, specificity and image resolution [17]. This non-invasive, irradiation-free, non-contact and painless technique does not provide information on morphological characteristics, but on functional, thermal and vascular changes in the tissue [14, 18-21].

It might be applied in the diagnosis of different diseases such as dermatitis, vascular dysfunctions, lesions in sensitive nerves, inflammatory processes and also in the monitoring of breast cancer [22-24]. However, there are still no studies which have comparatively documented the thermal coefficient variation in different phases of wound healing, especially when these are undergone to the action from the biomodulatory therapies already described.

The aim of the present study was to evaluate the effects of 670 nm laser photobiomodulation, ozone gas and ozonated oil on the pattern of thermal variation in the skin tissue of rats during repair, using infrared thermography.

Material and methods

Experimental study using an animal model

This study was forwarded to the Commission for Ethics in Animal Use (CEUA) with register number 67/2019 and obtained a favorable opinion. The sample included 40 male Wistar rats with an average weight of 250 grams (g). The rats were housed in specific plastic cages in groups of five, with good lighting conditions (light/dark cycle of 12/12 h) under a temperature of ± 22-24 °C, and were fed a commercial diet (Nuvilab, Quimtia, Colombo-PR, Brazil) and water ad libitum.

Surgical procedure

The animals were weighed, anesthetized with 10% ketamine hydrochloride (Dopalen®, Sao Paulo, Brazil) 75 mg/ml and 2% xylazine hydrochloride (Anazedan, Sao Paulo, Brazil) 5 mg/ml, with dosages of 2mg/kg and 3mg/kg respectively. After verified the loss of flexion reflex obtained from the paw, dorsal trichotomy and antisepsis with povidine iodine (Rioquímica, São Paulo, Brazil) were performed. Then, a circular incision was made with the aid of a metallic scalpel, (Biopsy circular scalpel, Stiefel, Germany) with 6 mm in diameter to obtain a uniform and standardized wound, which was performed by a single duly calibrated operator.

Experimental groups

A sample size calculation was carried out to determine what was n required for the present study. Therefore, statistical significance levels of $p<0.05$ and a coefficient of variability of 20% were used, based on studies with a similar design to the present research that found an increase in collagen (primary outcome) greater than 30%. Animals were randomly allocated into 4 experimental groups of 10 rats each, as described below and were sacrificed on the 5th and 10th days (20 animals in each period) after beginning the tests.

Group 1 - Control Group (CG): did not receive any type of treatment.

Group 2 - Laser Group (LG): rats were subjected to 4 punctual applications of 1 J/cm2, with a total dosimetry of 4 J/cm2, per day of application. It was used an aluminum and gallium arsenide laser semiconductor device (AlGaAs, 9 mW, 670 nm, 0.031 W/cm2 diode laser) with continuous emission and active tip area of 0.28 cm2 (VR-Laser), KC-610- Dentoflex, Brazil). The final dosimetry was 12 J/cm2 since the rats were irradiated for 3 consecutive days after the surgical procedure.

Group 3 - Ozone Gas Group (GG): rats received gaseous ozone therapy through subepithelial insufflation. The ozone was produced by the Philozon® generator (Philozon - Indústria e Comércio de ozone generators - LTDA, Santa Catarina, Brazil) at a concentration of 13?g/mL of ozone, from medical oxygen, with a constant flow of 1L/min. The mixture with oxygen was captured in a 5mL syringe and by using an insulin needle, the gas was insufflated at the edge of the lesion with a volume of 1mL in each application. This procedure was repeated for three consecutive days after the surgery.

Group 4 - Ozonated Oil (OG): rats was treated with 100% ozonized sunflower oil at 100% concentration (Philozon - Indústria e Comércio de Geradores de Ozone - LTDA, Santa Catarina, Brazil) dripped onto the lesion surface. For three consecutive days 50 µl was applied to the surface of the lesion in the same manner as in the previous groups.

The animals were submitted to biomodulatory therapies on days 1, 2 and 3 of the study. Each group with 10 animals had half of them sacrificed on the 5th day and the other half on the 10th day.

Infrared Thermography evaluation

The infrared camera used was the FLIR T430sc, focal plane array 320×240 pixel and image frequency of 30 Hz. The equipment has an 18 mm FOL lens, carries out studies in the temperature range of - 40 °C to 650°C, had an emissivity set to 0.98 and thermal sensitivity (NETD.) less than 30mK. It operates in the spectral range of electromagnetic waves between 7.5 and 13?m. The accuracy of the equipment is ±1°C or ±1% (limited range) ±2°C or 2%, whichever is greater, at 25°C nominal. Also, the time between switching the camera on and capturing the images was 15 minutes. The calibration of the camera is carried out using radiation sources that are traceable to International Standards at the Rede Brasileira de Calibração (Brazilia National Calibraton Satndards) FLIR Systems Brazil, Sorocaba, São Paulo, Brazil.

To obtain a pattern of images, the following protocol was adopted: the rats were placed in an orthostatic position on a fixed object 0.5 m in relation to the ground and acclimatized for a minimum period of 15 minutes before data collection. The camera distance from the animals was 0.5 m, and the temperature of the environment was controlled at around 20°C ± 1°C".

On the first day, rats were photographed at the following times: 10 minutes after shaving; immediately after the surgery and, immediately after therapy (except the control group). On the fifth and tenth days, animals of each group were anesthetized again and photographed with the thermographic camera 10 minutes after anesthesia. The normality and abnormality criteria adopted were based on studies by Uematsu [25,26], in which the pattern of clinical abnormality was established according to temperature variation. If the coefficient of thermal variation is greater than 0.3 the change is considered as significant.

Statistical analysis

To identify the association between the thermal variables, a database was created in Microsoft® Excel® 2010 (version 14.0.7132.5000), Microsoft® Office Professional Plus 2010, USA and analyzed in the R software (version 3.1.1). Data distribution for normality was tested using the Shapiro-Wilk test. The ANOVA test was used followed by the post hoc Bonferroni test. The significance level was $p<0.05$.

Results

The mean basal temperature of the rats was measured with a mercury thermometer introduced into the rectum and recorded at 33.2°C. Table 1 illustrates the temperature values of the animals at different moments during experiment. It was noticed the mean basal temperature of all rats was 33.2°C. After shaving, there was a raise in mean basal temperature to 34.3°C in the 40 animals participating of the experiment ($p<0.05$), with Delta T (ΔT) of +1.1°C. Right after the surgical procedure, there was a decrease in temperature in all rats, with a average of 33.7°C and delta T of -0.6°C ($p>0.05$).

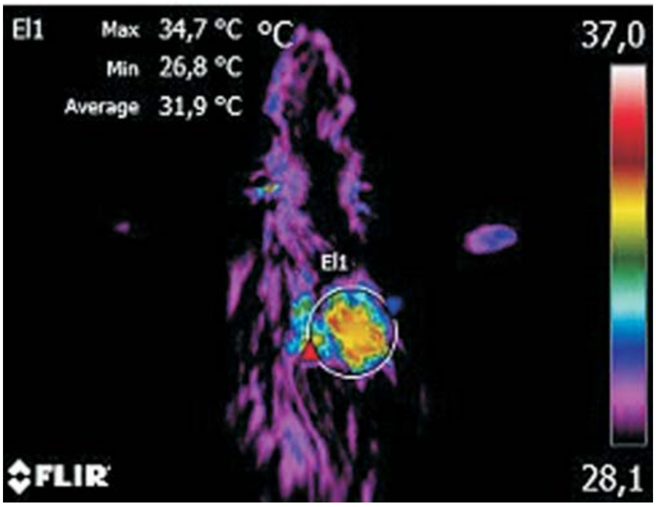

Figure 1:
Control Group (CG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 5 days.

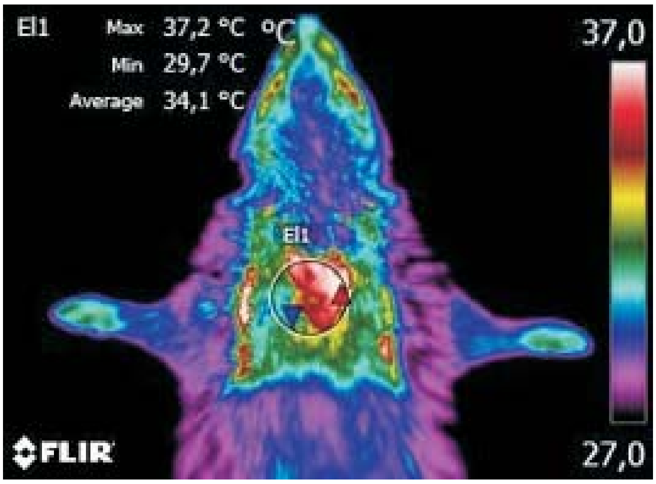

Figure 2
Gas Group (GG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 5 days.

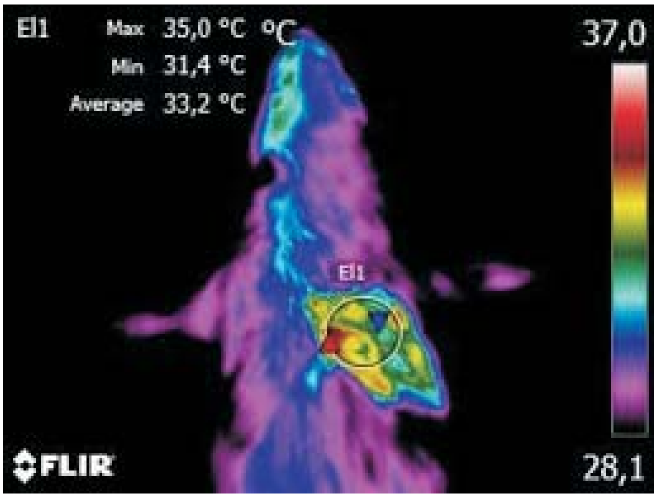

Figure 5:
Control Group (CG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 10 days.

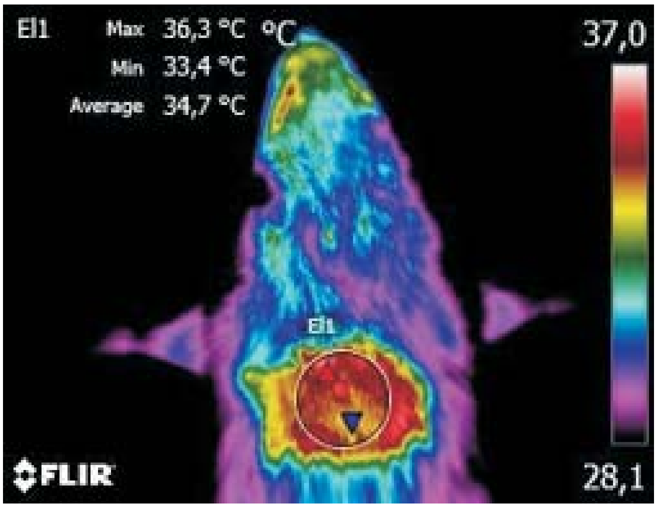

Figure 6
Gas Group (GG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 10 days.

Table 1:
Mean temperature with standard deviation of the experimental groups in different periods of the study.

EXPERIMENTAL GROUPS	**MEAN TEMPERATURE IN °C AND STANDARD DEVIATION**			
	POST SKIN INCISION	POST 1st THERAPY SESSION	5 DAYS	10 DAYS
CONTROL GROUP (CG)	33.7^{A}±0.8	-	35.7^{B}±1.0	33.3^{C}±1.1
OZONE GAS GROUP (GG)	33.7^{A} ±0.8	33.1^{D}±0.4	33.9^{E}±0.7	35.1^{F}±0.4
OZONATED OIL (OG)	33.7^{A} ±0.8	31.1^{G}±0.7	34.7^{H}±0.4	33.3^{I}±2.2
LASER GROUP (LG)	33.7^{A} ±0.8	32.7^{J}±0.6	35^{L}±0.7	33.7^{M}±1.3

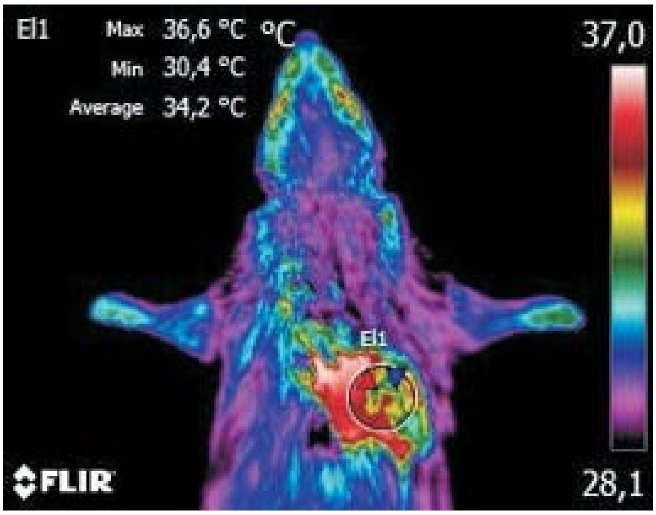

Figure 3
Ozone Group (OG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 5 days.

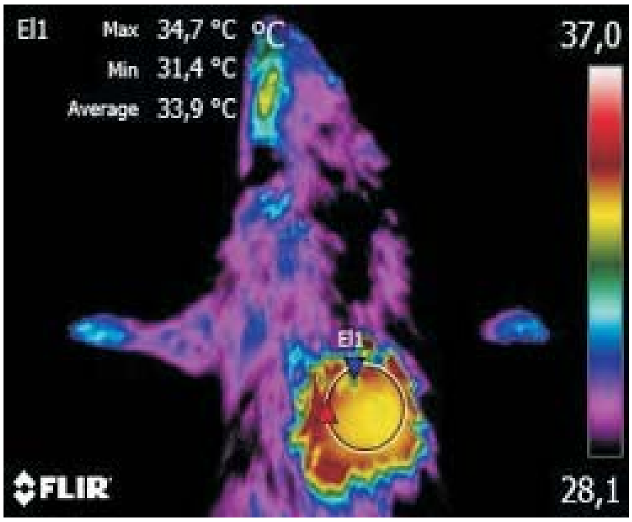

Figure 7
Ozone Group (OG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 10 days

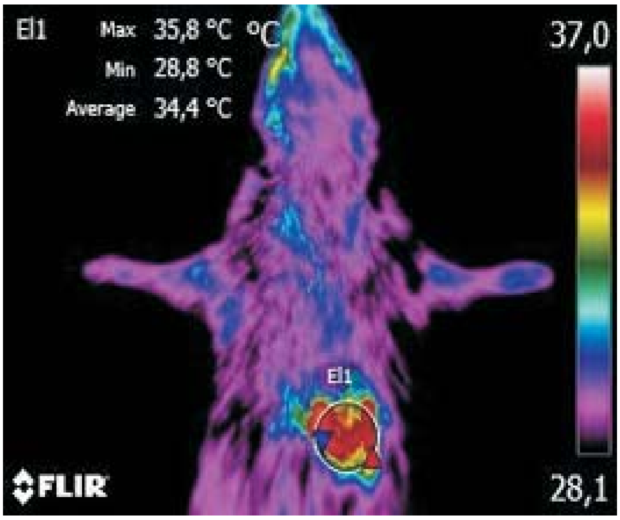

Figure 4
Laser Group (LG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 5 days.

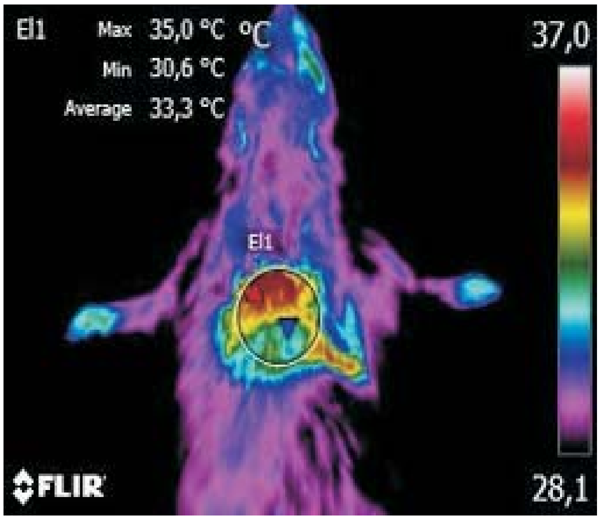

Figure 8
Laser Group (LG) animal thermogram showing maximum, mean, and minimum temperature in skin wound area, 10 days.

Table 2:
Intra and intergroup comparison of the thermal variation coefficient in the two periods of analysis.

EXPERI-MENTAL GROUPS	MEAN TEMPERATURE in °C											
	Post 1st Therapy Session				5 days				10 days			
	CG	GG	OG	LG	CG	GG	OG	LG	CG	GG	OG	LG
CONTROL GROUP (CG)	-	-0.6	-2.6*	-1.0*	-	-1.8*	-1.0*	-0.7*	-	+1.8*	0.0	+0.4
OZONE GAS GROUP (GG)	-0.6	-	-2.0*	-1.6*	-1.8*	-	+0.8	+1.1*	+1.8*	-	-1.8*	-1.4*
OZONATED OIL (OG)	-2.6*	-2.0*	-	-1.6*	-1.0*	+0.8	-	+0.3	0.0	-1.8*	-	+0.4
LASER GROUP (LG)	-1.0*	0.4	-1.6*	-	-0.7*	+1.1*	+0.3	-	+0.4	-1.4*	+0.4	-

*ANOVA, statistically significant difference ($p<0.05$) between CG/GG, CG/OG, CG/LG, GG/OG, GG/LG, OG/GG e OG/LG, just after fist therapy session, after 5 days, and after 10 days.

Once it was defined that the mean temperature to be adopted as a basis for the evaluation of biomodulatory therapies corresponded to that calculated after cutaneous surgery, the results of each experimental group are described below.

A. Control Group

At the end of the 5th day, the mean temperature was 35.7°C, with a temperature increase of around +2.0°C (ΔT=+2.0°C; $p<0.05$). On the 10th day, the average temperature was 33.3°C. The thermal coefficient variation was negative in this group with ΔT of -2.4°C ($p<0.05$); (Figures 1 and 5).

B. Ozone Gas Group

It was found that immediately after the use of ozone gas, the average temperature of the treated animals dropped to 33.1°C, with a ?T of -0.6°C ($p<0.05$). At the end of the 5th day, the mean temperature was 33.9°C, with a temperature increase of around +0.8°C ($p>0.05$). Regarding this group, around the 10th day the mean temperature was 35.1°C. The variation of the thermal coefficient was ascending in this group, for this period (ΔT =+1.2°C; $p<0.05$); (Figures 2 and 6).

C. Ozonated Oil Group

It was verified that soon after using the ozonized oil, the average local temperature of the treated animals dropped to 31.1°C, with a delta T of -2.6°C ($p<0.05$). At the end of the fifth day, the average temperature was 34.7°C with a temperature increase of around +3.6°C ($p<0.05$). Around the 10th day, in the group treated with ozonized oil the mean temperature was 33.3°C. The thermal coefficient variation was negative (ΔT =-1.4°C; $p<0.05$); (Figures 3 and 7).

D. Laser Photobiomodulation Group

In this experimental group, it was noticed that the use of laser photobiomodulation right after the surgical procedure promoted a decrease in mean temperature compared to the postoperative period of around 32.7°C, with a delta T of -1.0°C ($p<0.05$). On the 5th day after starting therapy, the mean temperature at the wound site rose to 35°C, with a delta T of +2.3°C. Ten days after the initial procedures, the average temperature was 33.7°C (ΔT =-1.3°C; $p<0.05$); (Figures 4 and 8).

Table 1 comparatively illustrates the coefficients of thermal variation (ΔT) of the experimental groups in the different periods of the study. It was observed that in the immediate period after carrying out the 3 biomodulatory therapies, all animals showed negative delta T in relation to the Control Group (GG ΔT=-0.6°C; OG ΔT=-2.6°C and LG ΔT =-1.0°C); ($p<0.05$).

Five days after therapies has started, when the mean temperatures obtained in the experimental groups were compared to those in the Control Group, it was found that thermal coefficient variation was significantly negative for all treated groups, with the highest ΔT observed between the CG and the GG (CG/GG ΔT=-1.8°C; CG/OO ΔT=-1.0°C and CG/LΓ ΔT=-0.7°C); ($p<0.05$); (Table 2).

On the 10th day, it was verified that the mean temperatures of the OG and LG groups were close to those of the Control Group, and the delta T showed a few variation (CG/OG ΔT=0.0°C; CG/LG ΔT=-0.4 °C); ($p>0.05$). The Ozone Gas group exhibited an increase in mean temperature in relation to that of the control group and ascending delta T, with a statistically significant difference (CG/GG ΔT=+1.8°C); ($p<0.05$); (Table 2).

Discussion

This study was the first to document the pattern of thermal variation under the light of infrared thermography in the area corresponding to experimentally induced cutaneous wounds in rats, submitted to biomodulatory therapies with 670 nm laser photobiomodulation, ozone gas and ozonated oil. The effects induced by such therapeutic agents in different types of tissue have been described as potentially anti-inflammatory, analgesic and tissue repair stimulators [27-29]. On the other hand, infrared thermography utilization in experimental healing models has also been explored [30]; however, its use for chronological assessment of the thermal gradient observed during tissue repair associated with biomodulatory therapies is still scarce in the literature.

Infrared thermography is an imaging test that was first introduced in medicine by Lawson in 1956, initially intended for the auxiliary diagnosis of breast cancer [19]. The literature assures that thermograms provide information about possible dysfunctions and, therefore, could help in the diagnosis of pathological states [16]. With a camera containing infrared detectors, the thermogram tracks the thermal coefficients on the body surface defined from the heat dissipation. Thermal variation may be related to circulatory factors and suffer alterations due to variations in blood flow and volume [19,22,31]. Vascular changes registered by the thermogram in an injured region may help to identify the probable origin of pain. Infrared thermography is a technique that provides real-time images of the temperature of a surface, in a non-invasive, non-radioactive and painless way. Therefore, has no restrictions on its use, despite its applicability limitations on morphological characteristics [18,23,32,33].

In the present study, a significant boost in local temperature was observed through Infrared thermography in all rats shortly after the shaving procedure, which represented a physical injury that triggered inflammation of the subcutaneous tissue. It is known that changes occur in the physical-chemical composition of the tissue in the lesion microenvironment such as low oxygen tension, pH reduction, presence of reactive nitrogen and oxygen species. These reactions trigger the biosynthesis of chemical mediators that activate the cells involved in the tissue repair process, as well as the vasodilation of the capillary net [34].

Although trichotomy has caused a significant increase in temperature in all rats included in the study, thermograms were only performed after the required time for thermal equalization, which was 10 minutes. As all animals were submitted to the same hair removal procedures and surgical wound induction, it is believed that the thermal variation patterns found in this study may really be attributed to the biomodulatory used therapies.

As well as in other models that evaluated tissue repair, in this experimental model of cutaneous healing, the first events that occur in the injured tissue are vasoconstriction, in an attempt to contain bleeding and increased vascular permeability. It contributes to the transmigration of leukocytes to the wound microenvironment [35-37]. On the first day treating with biomodulatory therapies, there was a decline in mean temperatures regarding wound areas of the treated groups, with greater significance in the OG (Ozonated Oil Group). In addition to a possible anti-inflammatory effect, it is important to highlight that this apparent difference in OG may also be explained by the fact that the ozonized oil was kept cold to ensure the maintenance of ozone stability [29]. It is likely that the temperature equalization time for using the oil was insufficient for it to reach a temperature similar to that of the environment. GG and LG also showed a temperature reduction of 0.6°C and 1.0°C, respectively. The immediate anti-inflammatory action induced by these two biomodulatory therapies in the tissue has been described in the literature and may justify this result [38,39].

On the 5th day of the experiment, a growth in temperature was observed in all groups, especially in the CG. In this stage of tissue repair there is an increase in cell migration, extravasation of serum molecules, antibodies and proteins through the capillaries, which corresponds to the transition from the exudative to the proliferative phase in the connective tissue. This scenario it was possible by the increase in blood supply and vascular permeability [35]. This fact is essential to the formation of granulation tissue, responsible for new cells nutrition in the region under repair [13]. Although these tissue changes were not analyzed in this study, it might be noticed that, in the light of infrared thermo.-graphy, Delta T was lower in the groups submitted to biomodulatory therapies. There are two possible explanations for this thermographic finding. One of them is supported by the documented anti-inflammatory action of laser photobiomodulation and ozone [40]. Marchionni et al., in 2010 [27], demonstrated that low power laser irradiation (670nm) on the 5th day of healing was able to induce a lower degree of exudative inflammation, with a lower percentage of polymorphonuclear cells. Also, authors found that there was a greater presence of myofibroblasts and collagen biosynthesis as histological results showed faster healing for the 5-day period. Although this research focused only on thermoscopic and thermographic analysis, it is possible to suggest that in this phase of skin repair corresponding to the 5th day, the treated groups had already accelerated the healing process and proliferative phenomena were established in the tissue.

Thus, it could be hypothesized that there was a balance between the tissue biostimulating action and the potential anti-inflammatory effect of biomodulatory therapies. The present study has limitations since there is a need to correlate variations in thermal coefficients with morphological aspects. And it may be done through a histopathological study of the skin corresponding to cutaneous injuries to comparatively evaluate the temperature captured by the thermogram and tissue changes, such as the degree of inflammation and fibroblastic and endothelial proliferation.

In this study, the animals in the OG (Ozone Gas Group) were the ones that presented a temperature closer to the initial baseline, on the 5th postoperative day. In turn, the OG group also showed a lower result than the control and laser, although higher than the gas group. Based on studies that demonstrate the chronology of the repair process in rats, during this period, the healing process transits between granulation and collagen production to compensate for tissue loss. The modulation of the inflammatory response reflects the ability to reduce pro-inflammatory agents such as TNF-α, IL1-β and COX-2 in the lesion area, mediators related to the increase in temperature [30]. This fact could also explain the lower temperatures in the groups submitted to biomodulatory therapies shown in the 5th. day. Some research studies have shown that ozonized oil is efficient in modulating the inflammatory process, stimulating angiogenesis and promoting enzymatic reactions that favor oxygen metabolism, in order to improve skin wound healing [29,41]. In the present study, it was observed that the ozonized oil was able to change the thermal pattern in the wound area showing signals of better healing results. These quantitative and qualitative specifications could be better observed from the morphological study of the injured and treated areas in the study.

On the 10th day of the study, it was verified that the Control Group, oil and laser, registered temperatures close to the initial average temperature of the animals, basal temperature. This information indicates that in this period, the wounds of these groups of animals were already in an advanced healing process. However, Ozonated Gas Group showed a higher temperature value when compared to the other groups. There are reports that ozone produces systemic biological effects and not just local ones, and that it is able of interfering with cell activity for a long period of time, despite having a rapid initial reaction in tissues [42, 43].

The present research strongly suggests that using thermograms might be useful to qualitatively and quantitatively monitor the pattern of microcirculation during tissue repair, in a non-invasive and painless way. Additionally, it proved the action of the biomodulatory therapies applied in the skin tissue subjected to physical injury, through the thermal coefficient variation.

It should be noted that this study has some limitations, since the specific analysis of only two repair periods could have been expanded. As matter of fact, the authors agree that it would be useful to study the early period of wound repair and do encourage the development of new studies in this area. Additionally, it is necessary to analyze the histopathological changes occurring in the animals' skin tissue to verify the occurrence of the biological processes already mentioned, such as neoangiogenesis, fibroblastic proliferation and collagen biosynthesis. Other limitation of the present study was the investigation of just one single dose of each therapy. It would be also important to study different application doses of the therapies, so that their effectiveness in establishing a safe protocol for using these technologies can be proven. Even so, it is also advisable to carry out similar studies in humans and comparative analyzes between the thermographic image and the histological characteristics observed in the tissue.

And finally, the authors also recommend the development of studies that evaluate the bactericidal potential of the biomodulatory therapies used to correlate it with thermoscopic and thermographic changes that can be documented by infrared thermography.

Conclusion

Infrared thermography proved to be an effective tool for capturing temperature variations during all stages of the research. It was possible to notice that the biomodulatory therapies applied caused vascular sensitive changes to the thermogram. Analyzing the images allowed concluding that there was a significant thermal variation in the groups tested with the therapies in comparison to the control one. Overall, with thermal imaging, ozone gas stood out from the other groups since it showed lower mean average temperatures during the inflammatory phase of the repair and also for raising the average temperature on the 10th study day for the remodeling phase.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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