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Green LED light has anti-inflammatory effects on burns in rats

Maria H.C.V. Catão [a], *Roniery O. Costa* [a,*], *Cassiano F.W. Nonaka* [a], *Ricardo L.C. Albuquerque Junior* [b], *Ivna R.R.S. Costa* [c]

[a] *Postgraduate Program in Dentistry, State University of Paraíba, Campina Grande, PB Brazil*
[b] *Postgraduate Program in Health and Environment, Tiradentes University, Aracaju, SE Brazil*
[c] *Postgraduate Program in Natural Resources, Federal University of Campina Grande, Campina Grande, PB Brazil*

ARTICLE INFO

Article history:
Accepted 9 July 2015

Keywords:
Healing
Lasers
Burns
Rats

ABSTRACT

Purpose: The aim of this study was to evaluate the effects of green LED light on inflammatory cells in skin burns: a histological study in rats.
Methods: In this study, 40 rats were randomly divided into 2 groups: G1 – Control (CTR) and G2 – Green Led (LED). Immediately after injury, green light (60 J/cm^2, 10 s, λ_{520} at 550 nm) was applied in a timely manner in the four coinciding points of the wound angles and at each point, the amount of 60 J/cm^2 with a time of 10s was delivered, totaling 240 J/cm^2 per session with 24 h intervals until the day before animal sacrifice at 3, 7, 14 and 21 days with a lethal dose of intraperitoneal anesthetic.
Results: In the histological analysis, animals treated with green LED, from 7 days, showed a significant decrease ($p < 0.05$) in inflammatory cells when compared to control group.
Conclusions: Green LED light provides an anti-inflammatory effect on skin burns of rats.

1. Introduction

Burn is one of the most devastating injuries that can reach man and one of the common causes of morbidity, mortality and severe disability in the long term. In Europe, it is estimated a mortality rate ranging from 1.4% to 34% for patients hospitalized for burns [1]. In the United States, more than one million burns occur each year, and about 5000 of these injuries result in death [2]. In turn, 1 million cases of burns are reported in Brazil, according to the Brazilian Society of Burns, of which 200,000 are treated in outpatient clinics and 40,000 require hospitalization [3].

Many therapeutic options have been proposed in literature to minimize the adverse effects of burns [4]. Low-intensity laser has demonstrated beneficial effects on the healing process of burns, acting in the inflammatory infiltrate, reepithelialization, collagen formation and maturation, in addition to its analgesic and anti-inflammatory action [5–11]. However, there is still divergence among researchers [12–14].

Light emitting diodes (LEDs) are small robust devices that work with wavelength range, ranging from ultraviolet to infrared spectrum [15] and are used in dentistry [16,17]. However, its effects on the healing process of incisions [18,19] and burns [20–22] have been recently studied. The aim of this study was to evaluate through histological analysis the effects of green LED light on inflammatory cells (IC) on burns in rats.

* *Corresponding author at*: Postgraduate Program in Dentistry, State University of Paraíba. R. Baraúnas, 351, Bodoncongó, Campina Grande, PB, CEP 58429-500, Brazil. Tel.: +55 83 3315 3300.
E-mail address: ronierycosta07@gmail.com (R.O. Costa).
http://dx.doi.org/10.1016/j.burns.2015.07.003
0305-4179/

2. Materials and methods

2.1. Animals

After approval by the Ethics Committee on the use of Animals (CEUA)/CESED, No. 0019/240712, 40 male adult Wistar rats were selected, with mass between 200 and 250 g from the animal facility of the Department of Medicine, Federal University of Campina Grande. The animals were kept in polypropylene cages coated with sawdust submitted to controlled temperature ($23 \pm 2\ ^{\circ}C$) and lighting (12 h light/dark cycle), with artificial food and water *ad libitum*. The animals were randomly divided into 2 groups: G1 – Control (CTR), G2 – Green Led (LED). Later, each group of 20 animals were divided into subgroups of 5 rats for each time (3, 7, 14 and 21 days) of observation.

2.2. Anesthesia/trichotomy/burn

The animals were anesthetized with a combination of 100 mg/kg of 10% ketamine and 5 mg/kg of 2% Xylazine applied in the intraperitoneally region. Soon after, the animal's back was shaved with a disposable razor blade associated with mild soap and water. Burn induction was performed on the back of animals with an iron instrument measuring 1.0 cm × 1.0 cm × 1.0 cm. This instrument was heated with the aid of torch until it was red. Heating was achieved with the blue torch flame in direct contact with the iron instrument for 40 s. When reached this state, the instrument was immediately put in contact alongside the animal's back, remaining for 20 s and burning the skin (Fig. 1).

2.3. Green light irradiation

Irradiation of the LED group (KONDORTECH, São Paulo, Brazil with 60 J/cm^2, 60 mW, 10 s, total energy 0.6 J and λ_{520} 550 nm/InGaN) was performed immediately after skin burn in a timely manner at four points coinciding with the wound angles. In this group, at each point, the amount of 60 J/cm^2 with a time of 10 s was deposited, totaling 240 J/cm ^{2} per session (Fig. 2). Applications were made daily, with the exception of animal's sacrifice day, in which no irradiation was performed.

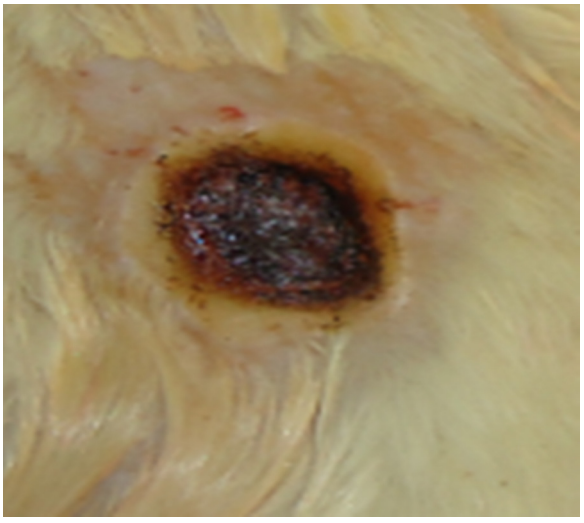

Fig. 1 – Aspect of burn in skin of rats.

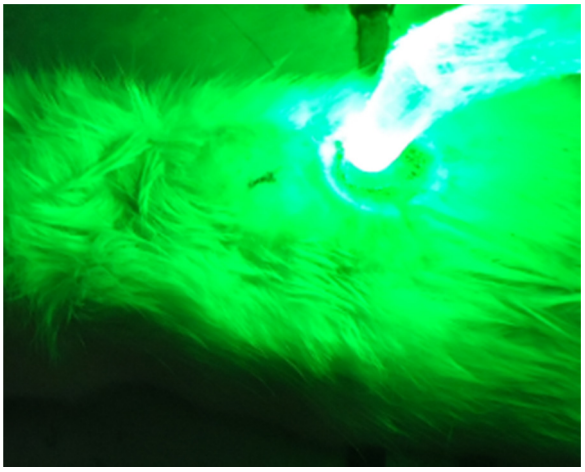

Fig. 2 – Green light irradiation in burn in skin of rats. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

2.4. Euthanasia/clinical evaluation/histological procedures

The animals were sacrificed by anesthetic association of 300 mg/kg of 10% ketamine (Cetamin®, Syntec, São Paulo, SP, Brazil) and 15 mg/kg of 2% Xylazine (Dopaser®, Syntec, São Paulo, SP, Brazil) injected into the peritoneum. Then, the specimen was removed using a scalpel and the wound was excised with a safety margin of 0.5 cm and fixed in 10% formalin. After the fixation period, the specimen was processed according to routine methods with Hematoxylin–Eosin (HE).

2.5. Quantitative analysis of inflammatory cells

Histological sections stained with H&E were used to quantify IC in CTR and LED groups within the same sacrifice time. For each slide at 400× magnification, counts of these cells in areas delimited by the wound were performed. The system consists of a CCD Sony DXC-101 video camera applied to an Olympus microscope CX31, from which the images were sent to a monitor (HP EliteDisplay E201). By means of a scanning system (Olympus C-7070 WideZoom), images were transferred to a computer (Intel™ Core i5) and processed using the ImageJ software, which provided the IC quantification.

2.6. Statistical analysis

Statistical analysis used the Shapiro–Wilk test to verify data normality, with 5% significance level for differences observed at $p < 0.05$. Statistical significance of the IC quantification was evaluated by nonparametric Mann–Whitney test.

3. Results

No significant difference in the number of IC in CTR (median 382) and LED groups (median 378) was observed in three days. In 7, 14 and 21 days, the LED group (median 372, 260 and 206, respectively) showed a decrease in inflammatory cells ($p < 0.05$) compared CTR group (median 390, 309 and 267, in that order) (Figs. 3 and 4).

In 3 and 7 days, groups (LED and CTR) revealed inflammatory infiltrate composed predominantly of neutrophils. After 14 days, groups showed inflammatory infiltrate composed of lymphocytes, macrophages, and neutrophils, the latter predominated on the surface areas of the wound. In 21 days, the groups showed inflammatory infiltrate composed of neutrophils, lymphocytes, and macrophages, with predominance of themononuclear component (Fig. 4).

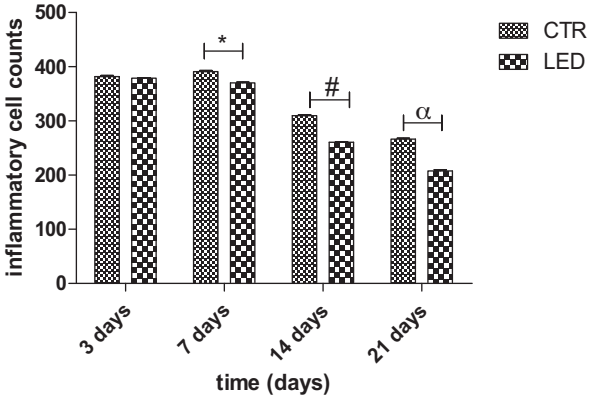

Fig. 3 – Count of inflammatory cells of LED and CTR groups at 3, 7, 14 and 21 days. Significant difference in 7 (*), 14 (#) and 21 (α) days ($p < 0.05$). Count of inflammatory cells × Time (days).

4. Discussion

Despite some disagreement among researchers [12,13,23,24], laser therapy has been proposed as one of the treatment modalities for soft tissue injuries, mainly due to its biostimulant properties that can speed up the healing process of burns [5,10,11,25].

In 7, 14 and 21 days, the LED group features its anti-inflammatory effect, since all animals were of the same gender, race, type and weight range, from the same place and were kept at the same temperature and lighting conditions. Histological studies have reported that laser therapy prevents the conversion of arachidonic acid into prostaglandin [26], by inhibiting the expression of enzyme cyclooxygenase 2 [27].

The results found in this study can be explained by the mechanism of action of LED on the skin, which has its effects on the lipid bilayer of the cell membrane, transferring energy through the rotation of the polarized extremities of lipids toward the light source [28], which can also stimulate fibroblasts to produce collagen, increasing mRNA expression of type-I procollagen [29].

Green LED light is used in dentistry, especially in esthetic restorations and its anti-inflammatory action on burning process is still controversial, as there are few studies in literature. Catão et al. [25] claim that green LED favors the healing process of burns in rats through its analgesic effects. However, there is controversy about the effectiveness of light

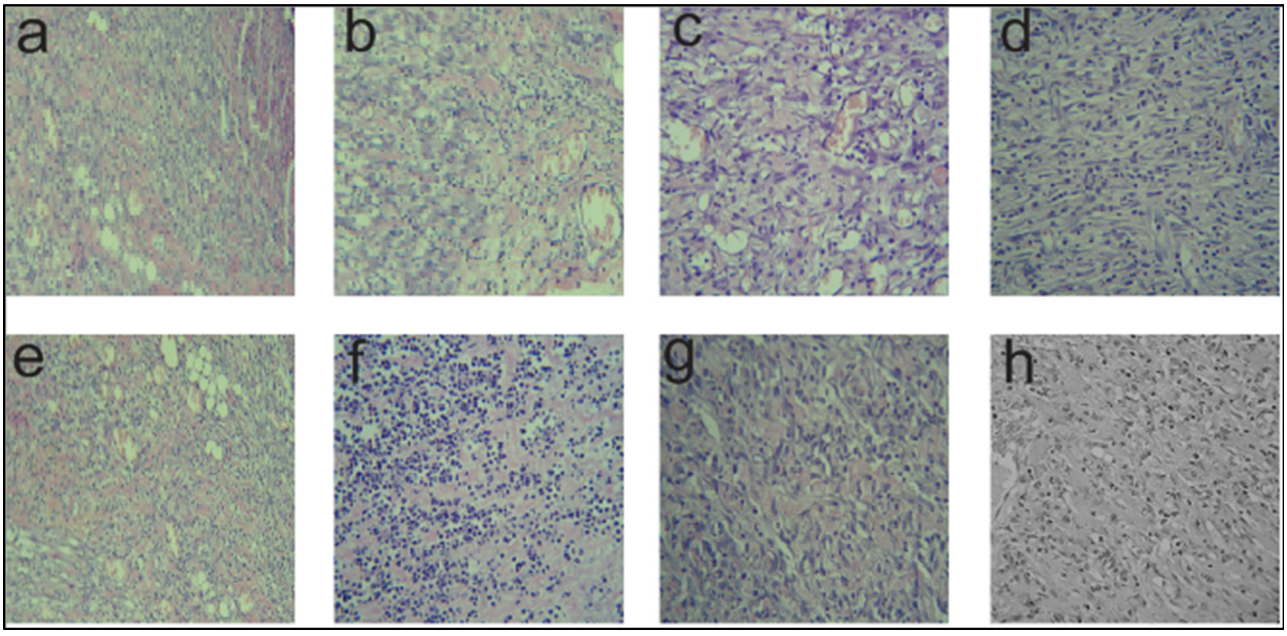

Fig. 4 – Histologic sections in HE (100×) of CTR (a, b, c, d) and LED groups (e, f, g and h) in 3 (a, e), 7 (b, f), 14 (c, g) and 21 days (d, h). Intense inflammatory infiltrate composed of neutrophils, lymphocytes and macrophages is observed in photomicrographs.

interaction (physical property) on tissues (optical properties) for a better understanding of the action of LED on skin burns [14,30].

5. Conclusion

The results of this study suggest that LED has anti-inflammatory effect on the skin burns of rats, favoring the healing process of burns. However, further studies should be carried out to better understand the effects of green light irradiation on burns.

Conflict of interest

None.

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