Healing activity of laser InGaAlP (660nm) in rats[1]

Atividade cicatrizante do laser AlGaInP (660nm) em ratos

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ABSTRACT

PURPOSE: To evaluate the effect the healing activity of diode laser Indium Gallium Aluminum Phosphorus (InGaAlP) ë660nm on healing of surgical wounds in rats.

METHODS: Fifty-four female Wistar rats were used, divided into three groups (n=18) and subdivided into three subgroups (n=6) to be studied in 5, 10 and 15th days after surgical procedures. The wound was induced in the dorsal-cervical using punch. The lesions were irradiated on alternate days with InGaAlP laser, the energy densities of 3J/cm^2 (L3) or 6J/cm^2 (L6). The control group received no irradiation. At 5, 10 and 15th days after surgery the animals were euthanized and the repair area was removed and histological sections were stained with hematoxylin-eosin and picrossírius. We evaluated macroscopic and histological lesions in the times cited, as well as morphometric analysis of angiogenesis and collagen content.

RESULTS: The wound healing activity InGaAlP laser was evidenced by increased angiogenesis group L3 and L6 in relation to control group (CG) at the 5th day (p=0.0001) and decreased polymorphonuclear infiltrate and hemorrhage (p=0.045 and p=0.07 respectively) in the groups L3 and L6 in relation to control group (GC). On the 10 and 15th days was also observed in groups treated with laser L3 and L6 stimulation was pronounced fibroplasia (p=0.0003 and p=0.034 respectively) when compared with the control group (CG).

CONCLUSION: The InGaAlP laser acted positively on the healing of skin wounds in rats.

Keywords: Wound Healing. Laser Therapy, Low Intensity. Rats.

RESUMO

OBJETIVO: Avaliar a atividade cicatrizante do laser diodo Alumínio Gálio Índio Fósforo (AlGaInP), ë660nm em feridas cutâneas induzida em ratos.

MÉTODOS: Foram utilizados 54 ratos *Wistar*, fêmeas, divididos em três grupos (n=18) e subdivididos em três subgrupos (n=6) animais para serem estudados no 5, 10 e 15° dias após o procedimento cirúrgico. A ferida foi induzida em região dorso-cervical utilizando *punch*. As lesões foram irradiadas em dias alternados com laser AlGaInP, nas densidades de energia de 3J/cm^2 (L3) ou 6J/cm^2(L6). O grupo controle não recebeu irradiação. No 5,10 e 15° dia após o procedimento cirúrgico os animais foram eutanasiados e a área de reparo foi removida e os cortes histológicos foram corados com hematoxilina-eosina e picrossírius. Foram avaliados aspectos macroscópicos e

histológicos das lesões nos tempos citados, bem como análises morfométricas da angiogênese e do conteúdo de colágeno. **RESULTADOS**: A atividade cicatrizante do laser AlGaInP foi evidenciada pelo aumento da angiogênese do grupo L3 e L6 em relação ao grupo controle (GC) no 5° dia (p=0,0001) e diminuição do infiltrado de polimorfonucleares e hemorragia (p=0,045 e p=0,007 respectivamente) dos grupos L3 e L6 em relação ao controle. No 10 e 15°dias observou-se também que nos grupos tratados com laser L3 e L6 houve estimulo acentuado a fibroplasia(p=0,0003 e p=0,034 respectivamente), quando comparados ao grupo controle (GC).
CONCLUSÃO: O laser AlGaInP atuou de forma positiva sobre a cicatrização de feridas cutâneas em ratos.
Descritores: Cicatrização. Terapia a Laser de Baixa Intensidade. Ratos.

Introduction

The healing of a wound consists of the reconstruction of the injured site in order to restore its integrity and normal function. It is a dynamic process where events are involved cellular, molecular and biochemical tests, which take place in a superimposed and interconnected. Changes in wound healing may present a high cost to society, and also decrease the quality of life for millions of people worldwide. However, attention has turned to the investigation of effective therapeutic strategies, and affordable alternatives that might work in this process[1]. The low intensity laser therapy (LLLT) is a generic term that defines the application of laser therapy with relatively low power (less than 500mW)[2]. Effects of low intensity laser on healing have been developed, checking analgesic, antiinflammatory and healing[3-5], although many studies don't show the specific type of laser used, refer only to such as laser 600nm[5]. The laser aluminum-gallium-indium-phosphorus (InGaAlP) has been widely used in LBI because of their power output of 30mW, which can reduce the stimulation of the irradiated site compared with other lasers such as helium-neon (He-Ne) and gallium arsenide (GaAs)[6].

However, being a relatively new type and widely used by health professionals, to waive calibration, it is painless and face handling, few studies have been performed on the same activity on the healing process, requiring standardization of methods and techniques. In this study, this paper the effect of InGaAlP laser was evaluated by the model of skin wounds induced in rats at days 5, 10 and 15th days after the induction of skin wounds.

Methods

The experimental protocol with the animals used in this study was approved by the Research Ethics Comitee of the Federal University of Goias, under the number 030/2010. Fifty-four female rats (*Rattus norvegicus albinus*), Wistar lineage, at 80 days of age, weight of 160-200g were used. Animals came from the Animal Colony of the Federal University of Goias (UFG), Goiania-GO, Brazil. Animals were adapted at the Experimental Surgery of the Center of Studies and Research Poisoning (CSRP), Pharmacy of School, UFG, for fifteen days, and kept in individual polyethylene cages covered with coarse sawdust, under controled environmental conditions (temperature at 23±2ºC, relative humidity of air at 50-70%, and 12-hour-light/dark photoperiod). Water and ration were supplied *ad libitum*.

The animals were weighed and divided into three groups with 18 rats. Subsequently, rats separated into three subgroups of 6 animals each, which were randomly assigned to one of the three treatment groups:

- Control Group (GC) non-irradiated, L3 and L6 photoirradiated groups euthanized 5 days after surgical procedures.
- Control Group (GC) non-irradiated, L3 and L6 photoirradiated groups euthanized 10 days after surgical procedures.
- Control Group (GC) non-irradiated, L3 and L6 photoirradiated groups euthanized 15 days after surgical procedures.

This study surgical procedures as described protocol adapted by Lopes *et al*.[7] and Garros *et al*.[8].

For the induction of wound used a circular metal punch of 1cm in diameter on the dorsal-neck of each animal. Anesthesia consisted of the administration, intramuscularly, the combination of ketamine and xylazine at doses of 70mg/kg and 10mg/kg, respectively, as model modified by Parente *et al*.[9].

Laser therapy application

Each animal in the treated subgroups received daily transcutaneous InGaAlP laser diode irradiation (IBRAMED - Laserpulse) application during 5, 10 and 15 days, for 1min with continuous output, potency of 30mW, spot area of 0.02 cm^2, wavelength (λ) 660nm (visible red), continuous mode, the energy density of 3J/cm^2 and 6J/cm^2. Laser array was positioned directly over the animal at a vertical distance of 1cm from the edge of the wound. The irradiation technique was carried out continuous and timely manner (cross-shaped) at the edge and sweeping in the wound bed. After irradiation, 0.1 mL was instilled saline into the wounds of animals of all groups. After 24 hours of surgical procedures, photoirradiated on wounds began at the same time and held by the same investigator, on alternate days until the day of euthanasia.

Macroscopic analysis

All animals were examined daily for general appearance, at which time they proceeded to macroscopic evaluation of the wound, observing the presence or absence of bleeding, exudate and crust, and the data recorded on individual sheets. For the morphometric analysis, the wounds were photographed on zero, five, 10 and 15th days after surgery using a digital camera mounted on a tripod, kept at a constant distance of 15cm from the wound, and then the images were digitized on a microcomputer with the aid of Image J Software 1.3.1 (NIH, USA).

Histological and morphometric analysis

After the sacrifice of the animals the wound area was surgically removed, fixed in buffered 10% formalin and paraffin embedded. Subsequently the preparation of histological slides serial 4ìm sections were obtained and stained in hematoxylin-eosin (HE) photomicrographs, the morphometric analysis and digital planimetry by point counts were performed by the same researcher. The number of fields in each sample photographed for morphometric analysis and digital planimetry by point counting was determined by cumulative average. Moreover, the slides also underwent picrossírius[10], technique for quantification of collagen under polarized light, calculating the percentage of area marked in green or reddish-yellow by field, using the software Image J 1.3.1 (NIH, USA).

Counting the number of blood vessels in the skin tissue was performed using planimetry by point counting with the aid of Image J software 1.3.1 (NIH, USA). A square lattice composed of 25 points was superimposed on the histological picture and the vessels were counted only at intersections present in the visual field, as the models proposed by Ribatti *et al*.[11] and Prado *et al*.[12]

The histological analysis was realized 5, 10 and 15th days after surgical procedures, were evaluated variables: fibrin, hemorrhage, hyperemia, inflammatory infiltration, reepithelialization epithelial hyperplasia. The results were visually classified according to the intensity found, and the data were transformed into quantitative variables, by assigning the following scores: absent (0), discrete (1 to 25%), moderate (25 to 50%), and accentuated (over 50%).

Statistical analysis

The results obtained were analyzed statistically using the GraphPad InStat (Version 3.5 for Windows). From the normality test of Kolmogorov-Smirnov test, parametric variables were evaluated by analyses of variance (ANOVA) and post - Tukey test and the nonparametric Kruskal-Wallis test and Dunn post-test. The significance level was $p<0.05$.

Results

Macroscopic evaluation

In this study, the surgical procedures and treatment were performed without complications. All animals recovered well from anesthesia, showing good general health, physical activity and normal behavior for the species. Purulent exudate wasn't observed in any of the wounds. The wound area decreased gradually with the progress of the healing process in the three experimental groups.

The crusts began to form the 2nd day after surgery, while in the treated group L3 and L6 were more thick and dry in the control group (GC), where they were more wet. From the 10th day after surgical procedure were not observed the presence of crusts in the groups treated with laser energy density 3J/cm^2 (L3) and 6J/cm^2 (L6). In the control group, treated only with saline, were seen to be hyperemic and crusty. On the 15th day after surgery was observed complete healing of wounds in all experimental groups.

There were significant differences between the control group (GC) and L3 the 10th day after surgery, indicating that the laser energy density 3J/cm^2 acted positively in the contraction of skin wounds, benefiting the centripetal contraction (Figure 1). There was no significant difference between experimental groups at the 5 and 15th day after surgical procedures related contraction of skin wounds.

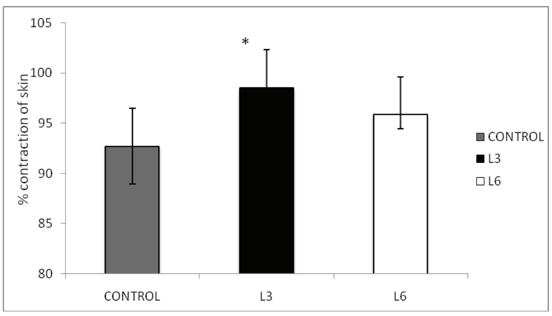

FIGURE 1 - Influence of processing of the control group L3 (laser diode InGaAlP 3J/cm^2) and L6 (laser diode InGaAlP 6J/cm^2) in the contraction of skin wounds in rats (%) on day 10 (ANOVA, Tukey post-test, p=0.003*). The vertical bars indicate the mean ± standard deviation of the averages.

Morphometric and histological assessment

In this study at the 5th day after surgical procedures the wounds were open and all wounds showed intensity moderate inflammatory infiltrate of polymorphonuclear (PMN). There were no significant differences between the histological parameters evaluated in this period. There was an increase in the number of blood vessels of L3 and L6 in the control group (Figure 2).

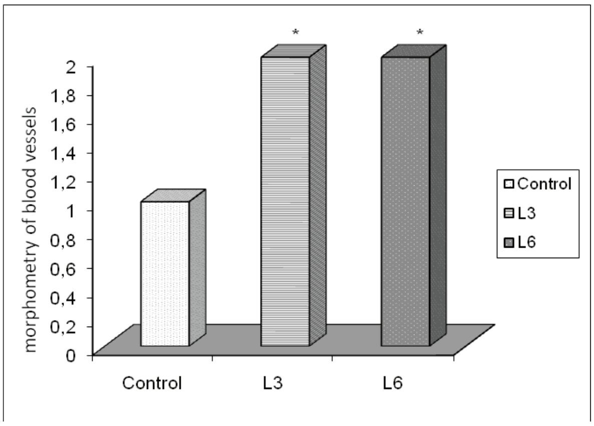

FIGURE 2 - Morphometry of blood vessels. Control (GC), L3 (laser diode InGaAlP 3J/cm^2) and L6 (laser diode InGaAlP 6J/cm^2) at the 5th day (Kruskal-Wallis, Dunn post-test, p=0.0001*). The vertical bars indicate the medians.

At the 10th day showed a decrease in the inflammatory process in all experimental groups. Significantly decreased as the bleeding in L3 compared to control (GC). Was also observed a significant decrease in the number of polymorphonuclear cells (Table 1). As for the content of collagen, we observed a significant increase in L3 and L6 in the control group, both in the histological analysis (Figure 3) and morphometry in picrossírius staining. This same result was observed at the 15th day between groups L3 and L6 compared to control (Figure 4)

TABLE 1 - Median of histologic at the 10th day after surgical procedures groups Control (GC), L3 (Laser 3J/cm^2) and L6 (Laser 6J/cm^2).

Histological Variables	Control (GC)	L3	L6	P
Fibrin	0^a	0^a	0^a	1.0
Hyperemia	1.0^a	1.0^a	1.0^a	0.26
Hemorrhage	1.0^a	0^b	0^b	0.007*
Inflammatory Infiltrate (PMN)	3.0^a	1.0^b	1.0^b	0045*
Collagen	1.0^a	2.0^b	2.0^b	00003*
Vascular proliferation	3.0^a	2.5^a	2.0^a	0.67

P=significance level. Different letters differ. (Kruskal-Wallis test, Dunn post-test, p<0.05*)

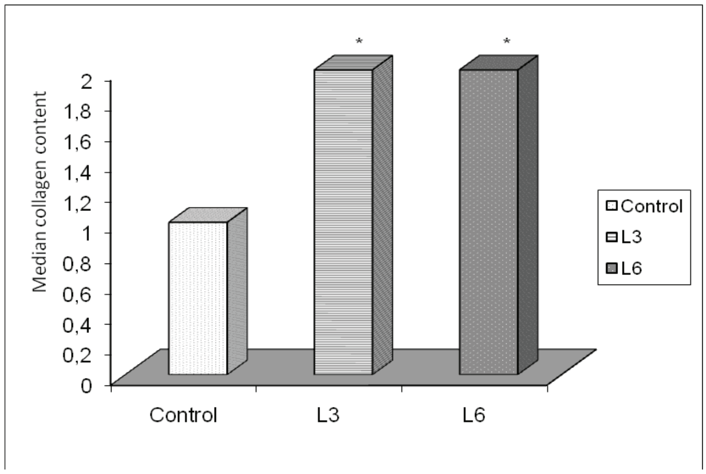

FIGURE 3 - Median collagen content at the10th day of the groups L3 (laser diode InGaAlP 3J/cm^2) and L6 (laser diode InGaAlP 6J/cm^2) compared to control (GC) (Kruskal-Wallis, Dunn post-test, p=0.045*). Vertical bars indicate the medians.

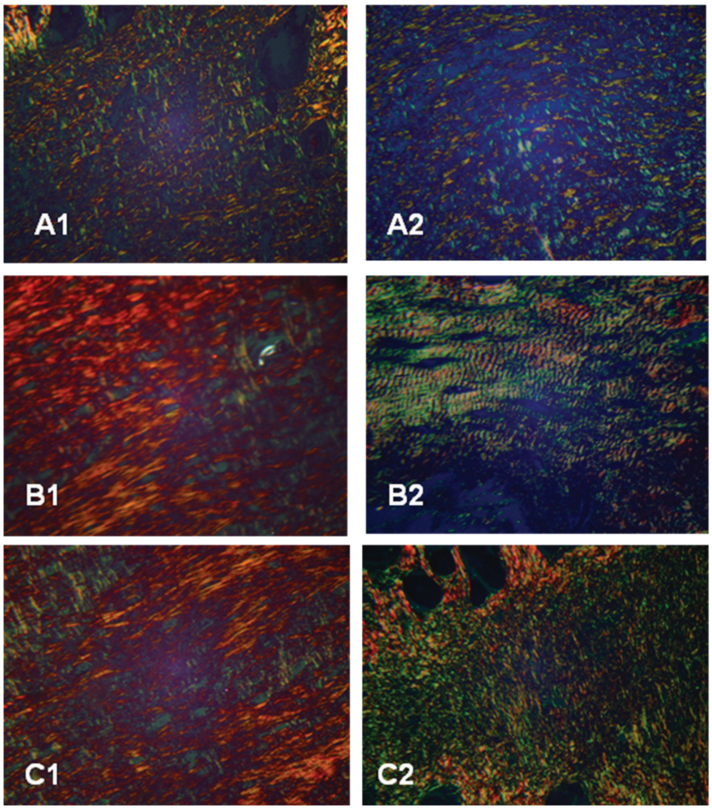

FIGURE 4 - Photomicrograph of skin wounds in rats at the 10th day (A1, B1, C1) and 15th day (A2, B2 and C2) after surgical procedures, showing collagen. Control GC (A), L3 (B) and L6 (C) respectively. Picrossírius. 200x.

Discussion

The increase in the contraction of the wound skin at the 10th day in groups submitted InGaAlP laser (660nm) indicated a positive effect on the healing process, the energy density of 3J/cm^2. Similar results were found in two other studies in rats and rabbits using the same energy density of[7,13]. However, the laser energy density that served helped the closure of skin wounds in a shorter period of time. Showed an increase in the number of blood vessels at the 5th day in those irradiated by laser. This finding is consistent with the results of the work of Walker *et al.*[14], which showed that the InGaAlP laser had a positive effect in neoformation of vessels, especially in the initial formation of granulation tissue. Admit that cells of the healing process respond the lasers that operate in the red spectral range, and this is mainly observed for the stimulation of angiogenesis[3]. In this study, neovascularization demonstrated is directly related to the activity of the laser on skin wounds.

There was a decrease in the number of polymorphonuclear and hemorrhage at the 10th day in groups treated with laser. It was shown that low intensity laser increased the number and activity of bacterium and on polymorphonuclear phagocytosis promoted more on the tissue debris. The photomodulation caused an initial increase in the number of inflammatory cells, which quickly removed the excess debris and then were reduced and was boosting production of growth factors necessary for its completion following[15]. The effects shown in this work as to hemorrhage and polymorphonuclear possibly occurred due to a positive effect of laser on the phase of inflammation. In this study both at the 10 and 15th days the increase in collagen content. It is assumed that the laser stimulates the transformation of fibroblasts in early myofibroblasts[6]. Araújo *et al.*[3] observed that the irradiated dermis showed a high number of fibroblasts, and these, we found a large amount of collagen vesicles and well developed endoplasmic reticulum, indicating increased activity compared to control group. Reddy[16] showed a 14% increase in collagen deposition in wounds of diabetic rats irradiated compared to non-irradiated. Byrnes *et al.*[17] studied the healing of wounds showing that the process was faster in animals irradiated with increased production of fibroblast growth factor (FGF). In this study, the result of an increased content of collagen in the wounds of irradiated animals are consistent with findings from previous studies[1,5,7,13] indicating the positive effect of laser on fibroplasia.

Conclusion

The laser therapy diode InGaAlP of energy density 3J/cm^2 and 6J/cm^2 had a positive effect on the healing of skin wounds induced in rats. Increased neovascularization, reduced inflammatory response, decrease of polymorphonuclear cells and hemorrhage, and stimulated fibroplasia.

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