Different LLLT Energy Levels on the Viability of Randon Skin Flaps in Rats

Ivaldo Esteves Junior, Igor Bordello Masson, Helio Plapler

Abstract— Background and Objectives: The skin flaps are still of concern to surgeons because failures can occur leading to necrosis of the same. Low intensity laser irradiation has been recommended as an effective tool for increasing the viability of ischemic flaps but its mechanisms of action are not yet precisely understood. We investigated the effect of low level laser irradiation (LLLT) on the viability of random skin flaps in rats by two different protocols. Methods: 24 Wistar EPM-1 rats were randomly located into three groups (n = 08). All animals went through a random dorsal skin flap measuring 10X4 cm. The sham group received only a simulated laser irradiation; group 0.29 was irradiated by a 20mW power laser, energy of 0.29 J on 12 points; group 7.3 received a 100mW laser irradiation, 7.30J on 12 points. These procedures were performed in the middle cranial flap and were repeated at three different postoperative times (POI, PO2 and PO1). The percentage of necrosis was measured on the seventh postoperative day through a paper template method. Results: The area of necrosis was smaller in group 7.3, compared to the sham and 0.29 groups (P<0.05). Groups 0.29 and sham showed no difference to each other. Conclusions: The low power level laser was effective in increasing the viability of random skin flap in rats at energy level of 7.30J.

Keywords— LLLT, skin flap, Dosimetry, Viability

I. INTRODUCTION

The skin flaps are widely used in reconstructive plastic surgery. Failures can follow, leading to the necrosis of the flap. The pathophysiological events that occur in the distal portion of the flap are not yet precisely understood. These events, which lead to decreased viability of the flap can be attributed to a number of extrinsic and intrinsic factors [1].

Many studies seek to increase the survival of the flap, trying to increase the blood supply, or decreasing ischemia [2-6], among these different resources used to enable the flap are if resources pharmacological and non pharmacological, including laser. For the latter, however, the studies do not explain the mechanisms of action of laser on skin flap, concerned only with macroscopic aspects of the area of viability [7-9].

The irradiation of low intensity laser has demonstrated therapeutic effects on different biological tissues, which can be either trophic (stimulating) or degenerative (inhibitory), depending on the form and amount of energy delivered to the target tissue [10]. The laser light has different wavelengths and may be visible or invisible. The visible laser light produces photochemical changes in the mitochondria through the stimulation of the respiratory chain [11,12]. The infrared laser light produces its photophysical effect on the cell membrane, probably in the Ca++ channels, increasing the collision between cells or simply causing a change of the cell membrane potential, which is reflected by the increase in ATP synthesis [13]. Both types of laser, visible and invisible, only provide power to trigger the metabolic processes required to restore a natural function of the cell or tissue. According to Almeida-Lopes [10], the action of low intensity laser is manifested in three ways, one of which acts directly into the cell producing an increase of the cellular metabolism and decreasing the cytokines and other inflammatory agents release [14 - 16].

This study aimed to investigate the effect of low intensity laser irradiation on viability of random skin flap in rats by two different dosimetric protocols.

II. METHODS

Animals and experimental model

This study was conducted in accordance to the Ethical Guidelines for Animal Experiments of the Council for International Organizations of Medical Sciences (CIOMS) and was approved by the Ethics Committee of the Federal University of São Paulo (1095/09).

Twenty-four Wistar EPM-1 male adult (12 weeks) rats weighing 260 to 320g were assigned randomly into three groups (n = 08). All animals were anesthetized with a peritoneal injection of tiletamine hydrochloride and zolazepam (25mg/kg). After epilation, a random skin flap measuring 10X4cm was built on the back of the animals, being nourished by a cranial vascular
pedicle [17]. A plastic barrier with the same dimensions was placed between the flap and its donor site [18, 19]. The flap was repositioned and sutured with simple stitches with 4-0 nylon monofilament.

**Laser irradiation**

After surgery, the *sham* group received simulated laser irradiation, the Group 0.29 was irradiated in 12 points (Figure 1) with energy of 0.29J per point, energy density of 10.36J/cm², power of 20mW, irradiance of 0.71W/cm² (total energy = 3.48J / animal), Group 7.3 was irradiated in 12 points as the previous group, with an energy of 7.3J, energy density of 260.71J/cm², power of 100mW, 3.57W/cm² irradiance (total energy = 87.6J / animal). The experimental groups were irradiated with a diode laser (Indium, Gallium, aluminum) with a wavelength of 660nm, beam area of 0.028cm² (Laser Quasar II Dentoflex®, Brazil). Off contact irradiation technique was used, and these procedures were done immediately after surgery and for the two subsequent days.

![Fig. 1. LLLT using punctual technique](image1)

A mask of the same dimensions of the flap containing 12 windows placed at 1cm on both sides and 1 cm between their central points (Figure 2) and occupying one half of this mask was applied over the flap. Irradiation began at the first window from the right top to the left and from the first row of to the last.

![Fig. 2. Template for standardization of laser irradiation with the same dimensions of the flap (10X4 cm)](image2)

**Analysis of necrosis**

The animals were killed on the seventh day after surgery by overdose of anesthetics and the percentage of necrotic area was calculated using the method of paper template [20]. The limit of viable tissue was characterized by soft, rosy and warm skin. Furthermore, the necrotic tissue was characterized by rigid, dark and cold skin, without fur (Figure 3).

![Fig. 3. Measuring the percentage of necrotic area. V= viable tissue and N= necrotic tissue.](image3)

These areas were demarcated in the animals and also on transparent paper with the aid of a precision scale. The following formula was applied for calculation:

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\text{Percentage of flap necrosis} = \frac{\text{Weight of paper template of flap necrosis}}{\text{Weight of paper template of total flap area}} \times 100
\]

**Statistical analysis**

Statistical analysis was performed by commercial software SPSS (SPSS ® 10.1 for Windows). Necrotic macroscopic findings were analyzed initially by non-parametric Kruskal-Wallis Test. When pointing out differences, Mann-Whitney
test was applied comparing data 2x2. P values <0.05 were considered statistically significant.

Results
Data concerning the percentage of necrotic area are presented below (Figure 4). The necrotic area was smaller in Group 7.3 when compared to the other groups. The sham group, though it did better than the sham group, showed no statistical difference to it regarding the necrotic area.

![Figure 4. Percentage of necrosis in the 3 groups](image)

Discussion
Skin flaps are being studied in different research centers [4, 9, 21-25]. This concern is due to the pathophysiological events that occasionally occur in the length of skin flaps. Kerrigan in 1983 [1] described such events as a result of this surgical technique widely used in reconstructive plastic surgery that can lead to necrosis of skin flaps.

Several studies assessed the effects of low intensity laser on the viability of skin flaps [4, 7-9, 25-32]. They used different experimental models with axial [4], [29] and random [7-9, 25, 30-32] flaps, but the model described by McFarlane et al. (1965) [17], developed for the study and prevention of necrosis was most used. These authors used different wavelengths in both visible (632.8 nm, 660 nm and 670 nm) [7, 25-27, 29-32] and in the infrared spectrum (780 nm and 830 nm) [4, 8, 9, 31, 32] The red laser light was the most used and also proved to be more effective [28, 32].

However, they all used different dosimetric parameters to get the energy doses. The potential means of several types of devices used ranged from 2.75 to 100 mW, times of application ranged from 2 to 600 s, energy densities between 0.19 and 185 J/cm², and finally deposited energies ranged from 0.0825 J to 3.6 J, so there is no standard and consensus of what would be the most suitable parameters to increase the viability of skin flaps. Smith et al. [26] and Cury et al. [31] showed that low-intensity laser was not able to reduce necrosis in their experimental models. Smith et al. [26] used little energy as 0.0825 J, and this energy was probably unable to stimulate the tissues meanwhile Cury et al. [31] used similar energy densities used by others [8, 9, 25, 32], even in the same experimental model, with different results. Comparing to Cury et al. [31] the number of stitches in its perimeter is higher than in other studies using the same model [7-9, 25, 30, 32]; the average stitches in this model was 23, less than the 36 made by Cury et al. [31]. We know that the tension on the flap can be a cause of local necrosis of skin flaps [1].

Our findings suggest a dose-dependence for this kind of experimental model, since the low intensity laser irradiation with energy of 7.3 J was effective in increasing the area of ischemic flap survival, unlike the group receiving 0.29 J of energy.

Further studies should follow to investigate how different doses of low intensity laser energy act on skin flaps in order to achieve a pattern of dosimetric parameters to improve the viability of skin flaps.

Conclusion
The low level laser was effective in increasing the viability of random skin flap in rats when used protocol with energy 7.30 J / point.

REFERENCES


