LASER 830NM ON THE VIABILITY OF SKIN FLAPS IN RATS SUBMITTED TO NICOTINE

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ABSTRACT

Objective: To investigate the possible effects of laser diode 830nm counter to the action of nicotine on the viability of the skin flap in rats. Methods: 24 Wistar-albino rats were used, divided into 3 groups of 8: Group 1 - subject to the surgical technique to obtain the random skin flap in cranial base, subcutaneous injection of nicotine of 2mg/Kg/day a week before and one week after surgery and simulation of laser. Group 2 - similar to group 1, the random skin flap in the cranial base were subjected to laser irradiation immediately after surgery and on the subsequent 4 days. After euthanasia, areas of necrosis and viable tissue were examined by a manual method (weighing the Role of Feedback) and by the semi-automated method of analysis (Mini-Mop®). Results: The results were statistically analyzed by ANOVA. The values of the percentage of necrotic area by the method of analysis showed a decreased area of necrosis in group 2 where the laser was applied. Conclusion: The 830nm laser was effective in improving the viability of skin flaps in rats subjected to the action of nicotine. Level of evidence II: Therapeutic Studies - Investigating the results of treatment.

Keywords: Laser therapy, low-level. Surgical flaps. Nicotine. Necrosis. Reconstructive Surgical Procedures.

Citation: *Neves LM, Marcolino AM, Prado RP, Thomazin JA. Laser 830NM on the viability of skin flaps in rats submitted to nicotine. Acta Ortop Bras. [online]. 2011;19(6):342-5. Available from URL: http://www.scielo.br/aob.*

INTRODUCTION

Traumatic hand lesions can cause extensive tissue loss from the cutaneous covering, exposing the underlying tissues, thus requiring skin flaps for the protection of these tissues[1]. These tissue losses can be corrected with procedures carried out in the area of reconstructive surgery, in which the surgeons use skin flaps as the most frequent resource in the performance of their interventions.[2,3] However, necrosis continues to be the main technical complication, and can lead to treatment failure.[4]
In literature, there are surveys using non-pharmacological therapeutic resources that merit special emphasis, such as acupuncture and electroacupunture[5] and polarized[6] and non-polarized low-frequency electrical currents.[5] Surveys using low intensity laser have appeared recently.[7]
In several experimental studies, various authors[8,9] investigated the cause and the deleterious effect of cigarette smoke or of nicotine separately. These studies, using different methodologies as regards the number and species of animals, type and time of exposure (smoke or nicotine), dosage of nicotine or of smoke, and type of surgery, observed a deleterious effect in the skin flaps exerted both by nicotine alone, and by cigarette smoke, causing an increase of the area of necrosis in the flap.
Today there are various studies that use drugs to minimize the deleterious effects of nicotine, thus decreasing the flap necrosis area.[8,9]
According to Prado et al.[7], low intensity laser therapy has been used to decrease the skin flap necrosis area in rats, as it is said to cause an increase in microcirculation and in vascular neoformation, favoring ischemic flaps.
Due to the shortage of studies using laser therapy to minimize the deleterious effects of nicotine, this study was carried out with low intensity laser therapy in an attempt to block these effects, bringing about an increase in the viability of the random skin flap in rats.

MATERIALS AND METHODS

Experimentation Animals

The study subjects were 16 male Wistar rats, weighing between 280 and 310 grams, and provided by the Central Vivarium of the Campus of the Universidade de São Paulo. The experimental procedures were conducted at the experimental morphology laboratory of the Department of Surgery and Anatomy of the Faculdade de Medicina de Ribeirão Preto-Universidade de São Paulo (FMRP-USP). The animals were maintained under standard

All the authors declare that there is no potential conflict of interest referring to this article.

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Article received on 5/20/10 and approved on 6/10/10.

vivarium conditions of 12hs of dark/light and temperature control (22-27°C), accommodated in individual polypropylene boxes, receiving commercial feed and water ad libitum.
The nicotine used in the experiment [Nicotine Sulfate L-1 Methyl-2 (3-Pyridyl) –Pyrrolidinesulfate; degree II; PM 422-6; SIGMA], was diluted with saline solution to the concentration of 1mg/ml[9] and injected in the subcutaneous tissues of the rats from groups 1 and 2, daily, one week before and one week after surgery, with a dose of 2mg/Kg.
This study was approved by the Committee of Ethics in Animal Experiments of FMRP-USP, protocol no. 048/2006.
The 16 rats were weighed and randomly distributed into two groups of eight animals. All the animals were submitted to the surgical procedure for harvesting of the cranial base skin flap, in a length of 10cm and width of 4cm on the animal's back.
Group 1 - submitted to the subcutaneous nicotine injection, once a day, one week before and one week after the surgical procedure, simulation of diode laser radiation.
Group 2 - submitted to the nicotine injection and 830nm diode laser radiation.
The application of low intensity laser occurred after the surgical procedure and on the 4 subsequent days.

Operating Technique

The animals were anesthetized intraperitoneally, with an equal association of ketamine (95mg/Kg) and xylazine (12mg/Kg). After anesthesia, the animals' backs underwent digital depilation. Soon afterwards the surgeon cut a cranial base flap measuring 10cm in length and 4cm in width on the animals' back. The flap was limited by a line that joins the lower angles of the scapulae and the upper edges of the pelvic girdle bones.[10]
The flaps were raised from the superficial fascia of the skeletal muscles and contained the skin and the subcutaneous tissue. A plastic (polyester/polyethylene) barrier with the same dimensions (10x4) was placed between the flap and the donor site (Figure 1), preventing revascularization of the flap by means of the donor site vessels.[11,12] After this, the flap was sutured with single stitches using 4-0 monofilament nylon thread in the same original position ("donor area"), separated with the distance of 1cm between stitches.[5]

Laser Radiation Emission Equipment

The participants used an Ibramed, AsGaAl (Gallium-Arsenide-Aluminum) diode laser, with a wavelength of 830nm, power of 30mW, beam area of 0.07cm^2 and continuous emission, manufactured by Indústria Brasileira de Equipamentos Médicos Ltda®. The skin flaps received the diode laser radiation at a point 2.5cm above the cranial base of the flap, with the help of a plastic mold, to standardize the radiation site. (Figure 1)
The radiation was always executed at the same time, using the point application with contact technique, fluency of 36J/cm^2, energy of 2,52J, duration of 84 seconds and with the laser positioned at 90° in relation to the skin flap.

Flap Necrosis Area Percentage Determination Method

Manual Method (Paper Template)

The percentage of necrosis area of the flaps was verified, on the seventh postoperative day, by means of the paper template method.[13]

Figure 1. *Laser application site (point technique with contact).*

A mold was drawn of the entire flap with delimitation of the necrotic area and the viable area on transparent tracing paper (Figure 2), which was then cut out and weighed on precision scales (error of +/- 0.0001g). Only the area corresponding to the necrosis of the flap that was also measured was cut out from this mold. The data thus obtained were applied to the following formula:

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

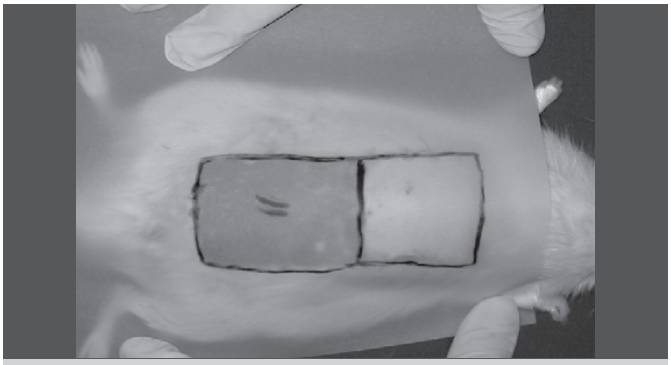

Figure 2. *Mold of the whole skin flap with delimitation between the necrotic area and the viable area.*

Semiautomatic Image Analysis Method (Mini-Mop®)

The percentage of necrosis area of the flaps was also verified, on the 7th postoperative day, by means of a semi-automatic image analysis method from the company Kontron Bildanalysis®. (Figure 3) The mold of the whole flap was drawn on transparent tracing paper, delimiting the necrotic area from the viable area of the flap, then fixed on the tablet of the Mini-Mop, and the perimeter of the total and necrotic areas was outlined with a stylus. (Figure 4) With this procedure the Mini-Mop automatically measures the area in mm^2 and sends the data to a compatible IBM-PC microcomputer, where they are stored in the form of files to be used in the Excel program for descriptive statistical analysis.

Euthanasia of the Animals

After the delimitation of the skin flap, the animals, still anesthetized, were sacrificed with an overdose of anesthesia.

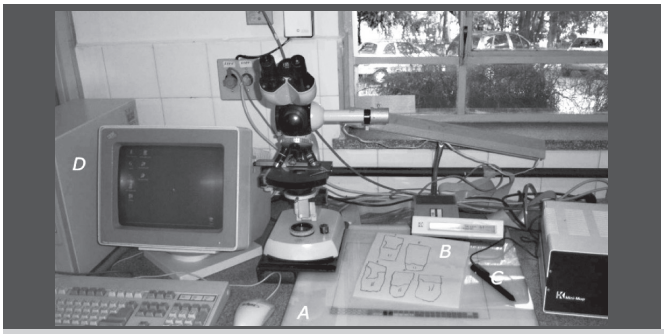

Figure 3. *Molds of the whole demarcated skin flap, fixed on the tablet of the Mini-Mop (A–tablet; B–demarcated molds; C–stylus; D–compatible IBM PC computer).*

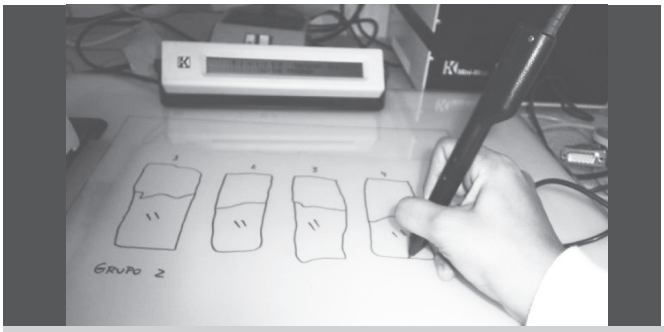

Figure 4. *Perimeter of the total area and necrotic area outlined with the stylus.*

Statistical Analysis

The data gathering was followed by a variance analysis (ANOVA) and multiple comparison post test - Orthogonal Contrast Method to compare the groups in each variable (Manual Method, Semiautomatic Method). The Intraclass Correlation Coefficient was applied with the intention of verifying the existence or nonexistence of statistically significant differences between the two methods.[14]

A significance level of 5% was considered for all the statistical tests performed.

RESULTS

The data obtained by descriptive statistical analysis in comparing the two methods used in the measurement of the areas where necrosis arose are presented in Table 1.

The intraclass correlation coefficient (ICC)[18] was applied to verify the existence or nonexistence of statistically significant differences between the two methods, weighing of the Paper Template and semiautomatic (Mini-Mop), considering both groups. The intraclass correlation coefficient obtained with a confidence interval of 95% indicated a substantial level of concordance between the methods. (Table 2)

The data obtained from the necrosis areas between the experimental groups in both methods were submitted to a Variance Analysis (ANOVA) and multiple comparison posttest (Orthogonal Contrast Method) and the results of this analysis with significance level of 5% are presented in Tables 3 and 4.

Experimental groups one and two presented statistically significant differences between one another in both methods ($p<0.05$).

Table 1. *Descriptive statistics of the percentages of the necrotic areas.*

GROUP	Method	N	Mean	SD
Nicotine	Semiautomatic	8	49.54	3.46
	Manual	8	51.07	2.27
Nicotine + Laser	Semiautomatic	8	39.23	14.46
	Manual	8	42.74	13.21

Table 2. *Categorizations for ICC24.*

Coefficient	Strength of concordance
below zero	*poor*
0.00 – 0.20	*slight*
0.21 – 0.40	*fair*
0.41 – 0.60	*moderate*
0.61 – 0.80	*substantial*
0.81 – 1.00	*almost perfect*

Table 3. *Comparisons between the groups for the variable weighing of Paper Template. Group 1–Nicotine; Group 2–Nicotine + laser.*

Comparison	p-value
Group 1 X Group 2	0.03

Table 4. *Comparisons between the groups for the variable measurement of the Paper Template (Mini-Mop). Group 1–Nicotine; Group 2–Nicotine + laser.*

Comparison	p-value
Group 1 X Group 2	0.02

DISCUSSION

Tissue loss after traumatic hand lesion can be corrected with procedures performed in the area of reconstructive surgery; for example, in the study by Gokrem et al.[2] they used the cross-finger technique for correction of loss of skin tissue in the digital pulp of the fingers.

Experimental studies[15] show that there is a deleterious effect of nicotine on skin flaps, causing an increase of the necrosis area. Several hypotheses are described involving the deleterious action of nicotine. According to Prigol et al.[16], it provokes the decrease of prostacyclin (PIG-2), which is the greatest inhibitor of agglomeration of platelets that have their dispersion reduced, facilitating thrombus formation. At the same time, it decreases prostaglandins and increases thromboxane A2, promoting greater adherence of platelets and inhibiting their dispersion.

Based on the ability of laser radiation to promote angiogenesis and an increase in local blood flow, some researchers have started to investigate the action of this therapeutic agent in the viability of skin flaps.[12,17]

In this study, the application of laser radiation occurred at a point located 2.5cm from the base of the flap, with a basis on the experimental model for low intensity laser in ischemic random skin flaps in rats, proposed by Prado et al.[7]

The experimental groups of this study underwent radiation for five consecutive days, that is, immediately after the operation and on the following 4 days. Pinfildi et al.[12] also made use of this methodology in submitting their experimental groups to radiation for five consecutive days.

In this study, the application technique employed was the point with contact method, similar to that used by Pinfildi et al.[12] and Amir et al.[17] There are studies that used lasers with different wavelengths and found significant results in the increase of skin flap viability.[12,17-19] Only a few studies that evaluated the action of the 830nm laser in random skin flap viability in rats were identified using different variables in the analysis.[11,19]

In this study, the fluency used was 36J/cm^2, based on the survey conducted by Prado et al.[11], who also used this dose, sufficient to obtain a decrease of the necrosis area of the flaps. In this study, with a wavelength of 830nm, total energy of 2.52J was applied on the flaps of the studied groups, the same energy applied in the study by Prado et al.[11], obtaining results with important reduction of necrosis area, even in animals submitted to the action of nicotine.

In this study the evaluation was performed on the seventh postoperative day as signs of necrosis of the flap appeared between three to four days, with final level of necrosis reached between seven and 10 days.[10]

The percentage of the skin flap necrosis area was evaluated using the paper template weighing method, initially proposed by Sasaki and Pang.[13] Besides its easy applicability, low cost and speed in the evaluation mode, this method has a small margin of error (<5%),[13] and is widely used in literature.[7,11,12]

This study also used a computerized semiautomatic method for image analysis (Mini-Mop®) to evaluate the percentage of skin flap necrosis area, by measuring the paper templates. It is an easily applicable method with fast evaluation mode and considerable precision of measurements, yet the cost is relatively high and there is only one account to date of its use in ischemic skin flaps.[20]

After the data was submitted to the statistical tests, it was demonstrated that experimental groups one (nicotine) and two (nicotine+laser) exhibited statistically significant differences between one another, in terms of necrosis percentage, in the Paper Template weighing and semiautomatic (Mini-Mop®) methods ($p<0.05$). This shows the major importance of laser therapy, promoting a decrease in the area of necrosis.

Aiming to verify the existence or nonexistence of statistically significant differences between the values obtained by a manual method (Paper Template weighing) and a semiautomatic method (Mini-Mop®), the participants applied the intraclass correlation coefficient (ICC)[14] considering both groups, and obtained the value of 0.64. This shows a substantial/high correlation between the two analysis methods, according to the categorizations for ICC.[14]

Surveys with low intensity laser have already produced a large number of studies; however, complete standardization of the parameters employed has not yet been achieved and the report of conflicting data hinders the comparison of results obtained. Therefore studies such as this one and other new studies are necessary to verify the importance and the dependence between each one of the parameters used in this type of laser therapy, improving its specificity and producing protocols with treatments that are increasingly safe and effective.

CONCLUSION

In the animals submitted to the controlled nicotine injection, low intensity laser radiation effectively decreased the areas of necrosis in the skin flaps, when compared to the areas of necrosis in the non-radiated animals.

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