Research Article

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Evaluation of Tissue Response in Sites Sutured with Cyanoacrylate and Submitted to Low Power Laser Therapy: A Randomized Clinical Trial

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Abstract

This study aimed to qualitatively evaluate the inflammatory response in sites sutured with nylon and 2-octyl cyanoacrylate and submitted to low-power laser therapy.

Background: 2-octyl cyanoacrylate acts as both a bonding agent for incisional wounds and a substitute for conventional suture methods after topical application, forming an occlusive layer that prevents the entry of any exogenous agent into the wound. Tissue reactions such as granuloma formation are cited in the literature as possible outcomes following 2-octyl cyanoacrylate use.

Methods: Thirty wistar rats, were randomized into groups of five, of which fifteen were sutured with 2-octyl cyanoacrylate and another fifteen with nylon thread. In the postoperative period, all the animals were submitted to low power laser therapy in a single centralised region, with light perpendicular to the surgical wound, applied every 48 hours via the ArGaAl laser, with a wavelength of 685 nm, a dose of 4 J/cm² and power level of 35mW. All the animals were kept in separate cages, under similar conditions, and provided with water and ad libitum feed. Animals were euthanized at 1 day (D1), 3 days (D2) and 7 days (D3). Tissue samples were removed from the central region of the wound and the slides were stained with hematoxylin and eosin.

Results: Histological sections were analysed by evaluating inflammatory parameters and in accordance with a previously standardized scale. Similar inflammatory reactions were observed between sutured wounds with nylon and cyanoacrylate, with no statistically significant differences observed.

Conclusions: Results suggest that there is no difference in the association between 2-octyl cyanoacrylate and laser therapy and nylon sutures with and the same As-Ga-Al laser application dosage.

Keywords: Wound healing, Low-level light therapy, Tissue repair

Introduction

The biocompatibility of 2-octyl cyanoacrylate with tissues has been described in the tissue repair process. Although

the literature demonstrates tissue reactions associated with cyanoacrylate use, there are no studies concerning the application of low-power laser therapy on wounds sutured with 2-octyl cyanoacrylate [1-5].

2-octyl cyanoacrylate is a biological glue, composed of a carbonic chain that can be used as a suture in regions where initial healing is expected to take place. The same occlusive layer covers the wound surface and extends to one centimetre above the edge of the incision [1-7].

Experimental studies where low power laser was used in the early stages of the tissue repair process demonstrate that it promotes cell proliferation. The light energy produced by the laser stimulates photoreceptors present in the cell nucleus, which encourages the synthesis of collagen, localised vasodilation, angiogenesis, fibroblast production and the production of T and B lymphocytes [8-12].

The aim of this study is to qualitatively evaluate the initial inflammatory response in rats submitted to surgical procedures sutured with nylon and 2-octyl cyanoacrylate and submitted to low power laser therapy.

Methods

The present sample consisted of 30 Wistar albino rats; clinically healthy male adults, approximately 180 days old and weighing an average of 300 g; sourced and kept at the laboratory of the Metropolitan Union of Education and Culture (Lauro de Freitas - BA). The samples were randomized.

During the experiment the animals were kept in individual polyethylene cages with regularly sanitized stainless steel lids at an ambient temperature of 23°C (+/- 1°C), in 12 hour alternating periods of light and dark, and with balanced ad libitum feeding and watering.

The rats used in the research were divided into two groups, according to the type of suture they were submitted to, and into a further three subgroups, according to observation period. All were irradiated with a low intensity laser. The postoperative observation periods were 1 day, 3 days and 7 days (Table 1).

Anaesthetic induction was carried out by administering Tiopental (1.25% solution). With the animal positioned in the ventral decubitus, an incision was made laterally, 1 cm in length and 1.5 cm from the end of the occipital bone, on epithelial and subcutaneous tissue. After exposure of the rats' dorsal muscular fascia, the suture was performed using monofilament black nylon thread (6-0) Mononylon® (Ethicon Inc.). The other group used 2-octyl cyanoacrylate glue (Dermabond®, Johnson & Johnson / Ethicon, Somerville, NJ).

Following the surgical procedure, the identified and numbered animals were returned to their respective cages and monitored daily throughout the observation period until the date of euthanasia. On completion of surgery, the animals received their first session of laser therapy (immediate application) at a dose of 4 J/cm². These were applied to a single centralized area on the suture, with an optical fiber of 0.028 cm². The equipment used was the Theralase system (supplied by DMC Equipamentos Ltd^a, São Carlos, SP, Brazil), at 685 nm, with a maximum power of 30 mW. The exposure time and energy density were calculated using the Tuner-Hode equation. The applications were repeated every 48 hours, during an experimental period of up to 7 days, following the same protocol proposed in the work carried out by Soares et al. (2008).

Following euthanasia, the each animal's dorsum region was removed and tissue specimens were processed using the paraffin inclusion method. Blocks were identified and submitted to microtomy. Sections of 5 µm were assigned to the routine Hematoxylin and Eosin histological staining technique in order to evaluate infiltration of edema, hyperemia, and polymorphonuclear and mononuclear infiltrates, as well as fibroblast counting, where the semi-quantitative analysis scale proposed by Medrado et al (2003) was used, with the following classifications: (0) absent, (1) discrete, (2) moderate, (3) intense [13].

Observations were tabulated in Excel and statistically analyzed. The Wilcoxon non-parametric test for paired measurements and the Mann-Whitney test were used for each observation period. A significance level of 5% was adopted and the R 3.3.0 program used for analysis.

Results

During observation, three animals underwent euthanasia outside the proposed observation period. These animals were distributed as follows: two animals, belonging to the cyanoacrylate group, were observed for seven days and one animal, belonging to the nylon group, was observed for three days.

In the statistical evaluation for the presence of hyperemia, the same reduction pattern was observed in the group sutured with cyanoacrylate according to number of observation days. When comparing the median values of the cyanoacrylate group with the nylon group, lower values of hyperemia were also observed, but none of the results presented a statistical difference, as evidenced in table 1.

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Evaluation of the edema present in the sample, as

Table 1: Qualitative evaluation of inflammatory response.

Variables	Time period					
	1 day		3 days		7 days	
	Median	q1-q3	Median	q1-q3	Median	q1-q3
Cyanoacrylate						
Hyperemia	1	1.0-1.0	1	0.0-1.0	0	0.0-0.0
Edema	1	1.0-1.5	1	0.5-1.0	1	0.0-1.0
Lymphocytes	1	0.5-1.0	1	0.0-1.0	1	0.0-1.0
Neutrophils	2	1.5-2.0	1	1.0-1.5	0	0.0-0.0
Nylon						
Hyperemia	1	0.5-1.0	1	0.25-1.0	1	0.0-1.0
Edema	1	1.0-1.0	1	1.0 - 1.0	0	0.0-0.5
Lymphocytes	0	0.0-1.0	1	0.25-1.0	1	0.5-1.0
Neutrophils	1	1.0-2.0	1.5	1.0-2.0	1	0.5-1.0

*p<0.05

seen in table 1, confirmed a reduction in edema in the two groups over the observation periods. However, no significant differences were found when we evaluated the observation periods within each group or between groups.

Regarding the presence of lymphocytes in the samples, we can see that in the cyanoacrylate group, even when the medians were maintained during the observation periods, there was a reduction in the number of lymphocytes in the intervals (Q1) representing 25% of the sample, although this was not statistically significant. In the nylon group, there was a pattern of increased lymphocyte count, but no statistical differences were verified when evaluating the medians according to time, or when associated with the cyanoacrylate group values (Table 1).

When evaluating the presence of neutrophils in the samples there was a verifiable reduction in the medians and quartiles in the cyanoacrylate group during the observation periods, where a median reduction of 2.0 to 0.0 was observed. However, no statistical differences were verified in the temporal analysis in the same group. In the nylon group, there was an increase in the median in the 3-day observation period, with a reduction in the sample evaluated at 7 days. However, no significant differences were observed in the temporal evaluation in this group or when associated with the cyanoacrylate group (Table 1).

In the quantitative analysis of fibroblasts, we observed that the fibroblast count in the Dermabond group was higher in the 24-hour period, with a p value of 0.016 and was statistically significant. In the other observation periods, no significant differences were observed when

compared with the total fibroblast count (Figure 1).

Figure 1: Analysis of fibroblast counts at sites sutured with Cyanoacrylate and Nylon.

Discussion

The study of tissue reaction has a direct contribution on the prediction of results. Planning, execution and adoption of precautionary measures are of great importance in surgical practice, in order to reduce inflammatory response, as is as the use of photodynamic therapy to modulate inflammation, promote analgesic effects and induce tissue repair [14].

The most intense inflammatory response observed in the initial phase is compatible with a reaction to tissue aggression caused by surgical procedure. The inflammatory infiltrate, as demonstrated by the presence of neutrophils, lymphocytes, hyperemia and edema in the samples collected from each group, corroborates other studies comparing laser irradiated groups [9,15].

Some studies have demonstrated the efficacy of using

sutures with adhesive compounds in surgery. These have the advantage of not reducing tissue perfusion caused by conventional suture and demonstrate inflammatory reactions similar to conventional or even minor suture materials in the final stages of inflammation [4,16,17]. In a comparative study between cyanoacrylate and nylon suture, cyanoacrylate has been shown to promote greater recruitment of polymorphonuclear leukocytes [18]. Another study compared the reaction to the absorbable suture thread with laser application. This demonstrated that photodynamic therapy was not appropriate for absorbable materials, since it promoted slower absorption of the material, which, in turn, triggered foreign body reactions during the observation period [14]. In this study, no differences were observed regarding the use of nylon or cyanoacrylate in the inflammatory response, with recruitment of lymphocytes, neutrophils, presence of edema and hyperemia.

Most authors in the literature demonstrate that laser therapy accelerates the tissue repair process by increasing cellular metabolism, even in tissues in a poor nutritional state, which induces the production of collagen, angiogenesis, fibroblasts and epithelial tissue. In relation to the laser irradiation protocol, they differ in the activation medium, the energy dose used, as well as the mode and the number of applications [8,11,19-23].

A great many irradiation protocols are found in the literature, with many different activating materials and wavelengths. This makes it difficult to compare results and subsequently select appropriate treatment parameters. In most cases, the lasers utilized are HeNe, AsGa, GaAlAs and InGaAlP [8,11,19,23-25].

A systematic review by Amid et al (2014) proposed that a low-power laser provides direct light to the cells, which results in the stimulation of molecules, without providing heat. A variety of responses were achieved, such as reduced inflammation, pain reduction, fibroblastic proliferation and collagen synthesis, but the literature does not demonstrate an identifiable protocol to be used for biomodulation, even with the use of a single laser [8,24,25].

A study conducted by Barbosa et al (2014) used the Arsenide Gallium and Aluminium laser with a wavelength of 830 nm and observed higher collagen synthesis in the irradiated animals, as did a study by Soares et al (2008), which used the same laser with a wavelength of 685 nm and also observed biomodulation in the inflammatory response [23,15]. A study conducted by Hussein et al (2011), using the ArGaAl laser with a wavelength of 890

nm, demonstrated a higher synthesis of collagen in the irradiated animals, lower inflammatory reaction and greater vascular proliferation. Alves et al (2013) also used the ArGaAl laser with a wavelength of 808 nm, but at 50 mW and 100 mW, and in different groups that presented osteoarthritis. This study confirmed that laser application at 50 mW was more effective in the reduction of IL-1 β and IL-6 (11), a result also observed by Huang et al (2012), who used the ArGaAl laser at 920 nm. A reduction of TNF and IL-1 was observed in the irradiated group [8]. In our study, the protocol proposed by Soares et al (2008) was used, and similar responses were observed in the two groups which had different suture materials: only the fibroblast count obtained a statistically significant difference in the first observation period when comparing the two groups.

The low power laser exerts biomodulatory effects on the inflammatory response. In this study (which used a dose of 4 J/cm² per application. It was not deemed necessary to include a control group to evaluate the difference between irradiated and non-irradiated animals, since it followed a protocol proposed by Soares et al (2008) that observes modulatory effects in the irradiated animals compared to control group. It is therefore assumed that, in our study, the laser was effective on both the nylon and the cyanoacrylate group. Other studies have used different dosimetry and obtained satisfactory results, such as that of Gonçalves et al (2010) which evaluated groups irradiated with 4 J/cm²; 30 J/cm² and 60 J/cm²; the results presented more deposition of type I collagen in the groups irradiated with 30 J/cm² and 4 J/cm². 9 Another protocol, using the helium and neon laser, with wavelengths of 632 nm and 1 J per application, observed differences in the proliferation of fibroblasts between irradiated and non-irradiated areas [25]. These studies corroborate the systematic review proposed by Khalid et al (2012), who observed that energy densities between 0.5 and 4 J/cm², as well as wavelengths of between 600 nm and 700 nm, induce cell proliferation, collagen synthesis and active photo-receptors in mitochondria. It should be noted, therefore, that there is no consensus in the literature in recommending a single protocol or even regarding the type of laser used for biomodulation.

Conclusions

Due to the reflective properties of cyanoacrylate after application, we suspected that alterations in laser penetration of the adjacent tissue would occur. However, the similarity in the results of the groups in all the periods we evaluated demonstrated that, in this study, cyanoacrylate did not prevent the dispersion and penetration of the laser in the studied parameters, or the laser was able to

establish similar inflammatory reaction in sutured wounds with both nylon and cyanoacrylate.

Conflicts of Interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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