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Potentiated anti-inflammatory effect of combined 780 nm and 660 nm low level laser therapy on the experimental laryngitis

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ABSTRACT

Reflux laryngitis is a common clinic complication of nasogastric intubation (NSGI). Since there is no report concerning the effects of low level laser therapy (LLLT) on reflux laryngitis, this study aimed to analyze the protective effect of single and combined therapies with low level laser at the doses of 2.1 J and 2.1 + 1.2 J with a total irradiation time of 30 s and 30 + 30 s, respectively, on a model of neurogenic reflux laryngitis. NSGI was performed in Wistar rats, assigned into groups: NGI (no treatment), NLT17.5 (single therapy), and NLT17.5/10.0 (combined therapy, applied sequentially). Additional non-intubated and non-irradiated rats were use as controls (CTR). Myeloperoxidase (MPO) activity was assessed by colorimetric method after the intubation period (on days 1, 3, 5, and 7), whereas paraffin-embedded laryngeal specimens were used to carry out histopathological analysis of the inflammatory response, granulation tissue, and collagen deposition 7 days after NSGI. Significant reduction in MPO activity ($p < 0.05$) and in the severity of the inflammatory response ($p < 0.05$), and improvement in the granulation tissue ($p < 0.05$) was observed in NLT17.5/10.0 group. Mast cells count was significantly decreased in NGI and NLT17.5 groups ($p < 0.001$), whereas no difference was observed between NLT17.5/10.0 and CTR groups ($p > 0.05$). NLT17.5/10.0 group also showed better collagenization pattern, in comparison to NGI and NLT17.5 groups. This study suggests that the combined therapy successfully modulated the inflammatory response and collagenization in experimental model of NSGI-induced neurogenic laryngitis.

1. Introduction

Nasogastric intubation (NSGI) is often used in clinical practice, during intensive care settings, emergency department, hospital wards, and even in home care [1]. However, several complications resultant of NSGI, such as laryngopharyngeal reflux disease and aspiration pneumonia, have been reported [1,2].

Laryngopharyngeal reflux disease is defined as retrograde reflux of gastric contents up to the laryngeal and pharyngeal level beyond the esophagus. Such disease potentially increase the risk of chronic cough, laryngitis, asthma, chronic obstructive pulmonary disease, recurrent pneumonia, hoarseness, difficulty swallowing, and laryngeal cancer [3].

Treatment for laryngitis depends on the pathogen or etiology, age, clinical features, and vocal demands of the individual. It can be treated with antibiotics, anti-inflammatory drugs (NSAID), oral and inhaled corticosteroids, proton pump inhibitors, antihistamines, and others [4]. However, current therapy of laryngitis may fail due to adverse aerodigestive side effects of drugs such as NSAID and corticosteroids, potentiating tissue injury during reflux laryngitis [5].

Despite laryngitis frequency in clinical practice, there are only a few experimental studies concerning the mechanisms underlying the laryngeal injury secondary to nasogastric intubation [2,6].

Technological advances led to the development of new equipment commonly used for diagnostic and therapeutic purposes, including lasers. Lasers are sources of electromagnetic radiation or light, having special properties that are different from other sources. The low level laser therapy (LLLT) has been used for the treatment of inflammatory diseases, with no remarkable side effects [7].

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Previous studies have suggested that LLLT can reduce the acute phase of the inflammatory response [8,9] and accelerate tissue repair in tendon and muscle injuries [9,10]. In addition, it has also been reported that LLLT can reduce carrageenan-induced paw edema, the expression of cyclo-oxygenase (COX)-2 and pro-inflammatory mediators, for instance tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6, in rats [11,12]. Other studies also demonstrate that LLLT alters the activity of inducible nitric oxide synthase (iNOS) [13] and stimulate the production of growth factors involved in the healing process, which includes platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β [8].

However, to the best of our knowledge, there are no experimental studies evaluating the use of low level laser in laryngitis secondary to nasogastric intubation. Therefore, this study aimed to investigate the effect of LLLT on the inflammatory process in a model of neurogenic reflux laryngitis.

2. Materials and methods

2.1. Animals

Male Wistar rats (350–450 g) were obtained from the Central Biotery of the Federal University of Sergipe (São Cristóvão, Brazil). Animals were randomly assigned into groups and maintained in plastic cages at controlled room temperature (21 ± 2 °C) with free access to food and water, under a 12 h light/dark cycle. All the experimental procedures were carried out during the light period of the day (08:00 a.m. to 05:00 p.m.) and complied with the guidelines on animal care of the Federal University of Sergipe Ethics Committee for Animal Use in Research (CEPA/UFS 34/11), which was conducted in accordance with the internationally accepted principles for laboratory animal use and care.

2.2. Experimental groups

Rats were randomly separated into four groups ($n = 6$/group): CTR – non-intubated rats; NGI – intubated rats; NLT17.5 – intubated rats treated with 17.5 J/cm^2 laser irradiation; and NLT17.5/10.0 – intubated rats treated with 17.5/10.0 J/cm^2 laser irradiation. CTR and NGI groups received simulated laser irradiation with the device turned off, in order to simulate the same stress in all the experimental groups. All the animals were submitted to subcutaneous administration of buprenorphine (0.05 mg/kg, 8/8 h, during the first 48 h) in order to minimize the pain.

2.3. Laryngitis model

For the induction of laryngitis, the animals were lightly anesthetized with isoflurane inhalation (1.5%, generated by a calibrated vaporizer) to allow swallowing reflex and were submitted to the nasogastric intubation procedure. Therefore, the distance from the nose to the stomach of each animal was measured in order to assess the mean length of the nasogastric tubes. Subsequently, a 10–13 cm narrow-bore nasogastric aspiration tube used for nasogastric intubation in premature newborns (Mark Med® no. 4) was inserted through the nasopharynx until it reached the stomach. The external tip was sutured to the nasal lateral cartilages (intubation, day 0) [2]. At the end of the intubation period (on days 1, 3, 5, and 7), the animals were anesthetized and euthanized with halothane (3%, inhaled), and perfused (NaCl 0.9% w/v containing heparin 0.1% v/v) through the ascending aorta. After confirmation that the nasogastric tube was still inserted in the stomach, the larynx was removed.

2.4. Low level laser therapy (LLLT) procedure

All the LLLT experimental procedures were carried out using a previously calibrated semi-conductor diode laser GaAlAs – 780 nm and InGaAlP – 660 nm (Twin Laser®, MMOptics, São Paulo, Brazil). The animals were submitted to laser transcutaneous irradiation by perpendicular contact to the region of the larynx. Laser arrays were applied in three points (22, 27, and 32 mm distant from the jaw) based on the preliminary findings, so that whole area of the larynx was covered by irradiation. The larynx region of all the animals was previously shaved, to provide better contact and absorption of laser light. LLLT-treated groups had a total of four irradiations sessions with 48 h interval between each laser application. The first session was performed 24 h after nasogastric intubation. The parameters of LLLT are described in Table 1.

2.5. Measurement of myeloperoxidase (MPO) activity

Additionally, MPO activity, a marker of neutrophil accumulation, was measured in the larynx, based on the method originally described by Bradley et al. [14]. Briefly, after the intubation period (1st, 3rd, 5th, and 7th day, 8 h after LLLT) the larynx was collected, weighed and homogenized (10 mg/mL) in phosphate buffer (50 mM, pH 6.0) containing 0.5% hexadecyl-trimethylammonium bromide. The homogenates were incubated (2 h at 60 °C) to inactivate endogenous catalases. The supernatants were mixed with o-dianisidine dihydrochloride (0.167 mg/mL, in 50 mM phosphate buffer) solution containing H_2O_2 (0.005%). Changes in absorbance of the resulting chromophore were read at 460 nm (Labsystem Multiskan, Helsinki, Finland). The results were expressed as units of MPO (UMPO)/mg tissue, where one UMPO is defined as the amount of enzyme that degrades 1 μmol of H_2O_2/min.

2.6. Histopathological procedures

The larynxes collected at day 7 were formalin-fixed and paraffin-embedded according to the routine laboratorial techniques. Subsequently, serial 5 μm thick sections were obtained and stained in hematoxylin–eosin, sirius red, and toluidine blue.

2.6.1. Assessment of inflammatory index

Histopathological sections stained in hematoxylin–eosin (HE) were used to the descriptive analysis of the inflammatory index. The intensity of the inflammatory response was assessed as follows: 1 (inflammatory cells representing less than 10% of the cell population observed within the wound area), 2 (inflammatory cells representing between 10% and 50% of the cell population observed within the wound area), and 3 (inflammatory cells representing

Table 1
Description of low level laser therapy parameters applied to the irradiated groups.

Parameters	NLT17.5	NLT17.5/10.0
Emission	Continuous mode	Continuous mode
Wavelength (nm)	780 ± 10	780 ± 10 + 660 ± 10[a]
Light emitter active medium	GaAlAs	GaAlAs + InGaAlP
Power output	70 mW	70 mW + 40 mW
Spot size	0.04 cm^2	0.04 cm^2
Power density	1.75 W/cm^2	1.75 W/cm^2 + 1.00 W/cm^2
Energy density	17.5 J/cm^2	17.5 J/cm^2 + 10.0 J/cm^2
Irradiation time (each point)	10 s	10 s + 10 s
Total irradiation time	30 s	30 s + 30 s
Total energy	2.1 J	2.1 J + 1.2 J
Beam divergence perpendicular to the junction	17°	17°
Tip angle	50°	50°

[a] The two wavelengths were applied sequentially.