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Interaction between low level laser therapy and anesthesia in wistar rats

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ABSTRACT. The aim was to assess whether the low level laser therapy (LLLT), 660 nm, can lower the effect of injectable anesthetics in rats. Wistar rats (n = 20) were used in two steps: 1) grip strength test and measuring the anesthesia time for control (G1A) and irradiated (G1AL) groups; 2) after 15 days, rats received new anesthesia injection and were evaluated for nociception (G2A - control; G2AL - laser). Anesthesia was induced by ketamine hydrochloride (75 mg kg^{-1}) and xylazine (10 mg kg^{-1}), by intraperitoneal injection, according to the body weight. LLLT used was 660 nm at four sites along the right hind limb. Anesthesia time was shorter for the G1AL ($p = 0.0031$). There were significant differences between pre- and post-intervention in the grip strength test ($p < 0.001$), but no differences were detected between groups ($p = 0.459$). For nociception, G2AL achieved higher values than G2A ($p = 0.019$ and $p = 0.032$), and also for the comparison between pre-injury values with the following values ($p < 0.001$) although no significant difference was found between 10 and 60 minutes ($p > 0.05$). It can be concluded that the LLLT caused no significant reduction on the effect of anesthesia.

Keywords: low-level laser therapy. anesthesia. pain measurement.

Interações entre laser de baixa potência e anestesia em ratos wistar

RESUMO. O objetivo deste estudo é avaliar se o laser de baixa potência (LBP) pode desempenhar algum efeito na diminuição da ação de anestésicos injetáveis. Foram utilizados 20 ratos Wistar, em duas etapas: 1) realizado teste de força de preensão e contagem de tempo de anestesia para animais controle (G1A) e irradiados (G1AL); 2) após 15 dias, novo procedimento anestésico e avaliada a nocicepção (G2A – controle; G2AL – laser). Foram utilizados os anestésicos Quetamina (75 mg kg^{-1}) e Xilazina (10 mg kg^{-1}), de acordo com o peso corporal, com injeção intraperitoneal. O laser utilizado foi 660 nm, em 4 locais, ao longo do membro pélvico direito. O tempo cronometrado de anestesia foi menor para G1AL ($p = 0,0031$). Na avaliação da força de preensão, houve diferenças significativas ($p < 0,001$) entre o momento pré x pós, mas não entre os grupos ($p = 0,459$). Para a nocicepção, G2AL obteve valores maiores do que G2A ($p = 0,019$ e $p = 0,032$), bem como ao comparar os valores pré-lesão com os seguintes ($p < 0,001$), mas sem diferenças entre 10 e 60 minutos ($p > 0,05$). Conclui-se que o LBP não apresentou grandes interações com relação à diminuição do efeito da anestesia.

Palavras-chave: terapia a laser de baixa intensidade. anestesia. medição da dor.

Introduction

Low level laser therapy (LLLT) has been widely used in clinical practice because it is not invasive, painless, easily administered (Bjordal et al., 2008), and has beneficial effects for the irradiated tissues, such as activation of microcirculation (Maegawa, Itoh, Hosokawa, Yaegashi, & Nishi, 2000), vasodilation production (Plass, Wieselthaler, Podesser, & Prusa, 2012) and neovascularization (Cury et al., 2013). LLLT modulates several biological processes, increasing mitochondrial respiration and ATP synthesis (Karu, 1999), changing enzymatic reactions by inhibiting the synthesis and release of prostaglandins (Marcos et al., 2011), besides producing analgesia via release of beta-endorphin (Meireles et al., 2012) and changes in nerve conduction (Chow, David, & Armati, 2007; Yan, Chow, & Armati, 2011).

In surgical procedures, an important component is the anesthesia. For experiments with small animals, it is commonly used a mixture of ketamine and xylazine as anesthetics. Ketamine has sympathomimetic action on the sympathetic nervous system (Brookes, Brown, & Reilly, 2002), increasing the cardiac output, blood pressure and pulse rate (Han, Kim, Joo, & Kim, 2012), in addition to vasoconstriction (Brookes, Brown, & Reilly, 2000). One the other hand, xylazine acts through the α2-adrenoceptor and mediates the vasoconstriction of smooth muscle and vessels, increasing peripheral vascular resistance and arterial and venous pressure in response to catecholamines (Haniuda, Itoh, & Chiba, 1989).

If anesthetics are injected into an area with large vascular activity, the anesthesic substance is rapidly absorbed, which produces a shorter duration of anesthesia time. Increased local circulation, such as vasodilation produced by LLLT, in both local and systemic way, can increase the uptake of anesthetic agents, reducing the action and duration of anesthesia and stimulating precocious pain in the post-operative period (Aras, Omezli, & Gungormus, 2010). The aim of this study was to assess whether the LLLT, 660 nm, can lower the effect of injectable anesthetics (ketamine associated with xylazine) in Wistar rats, considering that these animals and anesthetics are routinely used in experiments aimed at scientific maturity with a view to future applications in humans.

Material and methods

Animals and experimental groups

This study is quantitative and experimental, developed in two stages summarized in the flowchart below (Figure 1).

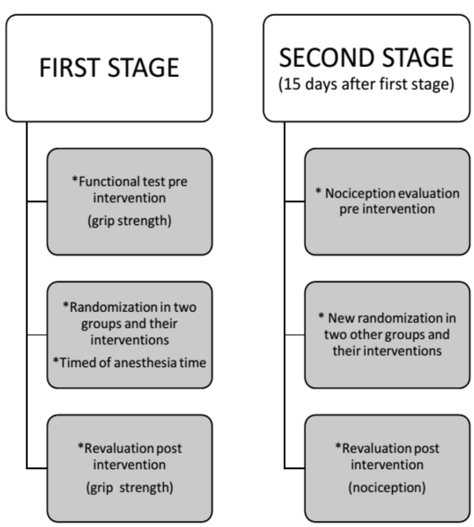

Figure1. Flowchart of the experimental design.

Male Wistar rats (n = 20) with 357.20 ± 40.21 g were obtained from the Central Animal Vivarium of the State University of West Paraná (UNIOESTE). The study was conducted according to international standards of ethics in animal experimentation and was approved by the UNIOESTE Ethics Committee on Animal Use (protocol 01112). Animals were grouped and housed in polypropylene cages, under controlled environmental conditions, with a 12 hours light/dark cycle, 23 ± 2ºC temperature, with free access to water and food.

In the first stage, it was applied pre-intervention functional test of grip strength to the right forelimb. Then, animals were randomly divided into one of two conditions: anesthetic procedure isolated or anesthetic procedure combined with LLLT applications:

- Anesthetic procedure isolated Group stage 1 (G1A, n = 10): animals in this group were anesthetized with ketamine hydrochloride (75 mg kg^{-1}) and xylazine hydrochloride (10 mg kg^{-1}), by intraperitoneal injection, according to the weight of the animals;
- Anesthetic procedure plus LLLT applications Group stage 1 (G1AL, n = 10): animals in this group were anesthetized similarly to G1AL and received application of 670 nm laser on 4 points in the right hind limb according to the protocol described in detail in a subsequent section.
- Anesthesia time was determined in this phase. A stopwatch was used to measure the time required for the animal to awake, performing limb and/or trunk movements. The post-intervention assessment of grip strength was conducted after awakening the animals.

In the second stage, animals underwent incision parallel to the fibers of the biceps femoris muscle to expose the right sciatic nerve. Animals were randomized again in two other new groups:

- Anesthetic procedure isolated Group stage 2 (G2A, n = 10): animals in this group were anesthetized with ketamine hydrochloride (75 mg kg^{-1}) and xylazine hydrochloride (10 mg kg^{-1}), by intraperitoneal injection, according to the weight of the animals;
- Anesthetic procedure plus LLLT applications Group stage 2 (G2AL, n = 10): animals of this group were anesthetized identically to G2AL and received application of 670nm laser with the same protocol used in the first stage.

Prior to this procedure, the animals underwent the test paw withdrawal threshold with stimuli in the incision area and on the plantar surface of the right paw (pre-assessment).

LLLT protocol

Soon after stunning the animals, by the absence of voluntary movements in limbs and trunk, there was application of LLLT, at 660 nm, 30 mW, 0.06 cm^2 spot. To this end, the animal was positioned in the left lateral decubitus position, being used in 4 locations, along the right pelvic limb, at the following points: the region of origin of the hamstring muscles, the thigh middle third, popliteal region and leg middle third. The fluence was 5 J cm^{-2} at each point, in a total of 20 J cm^{-2}, with energy of 1.2 J.

Evaluation of grip strength

For the assessment of grip strength, one meter grip strength was used (Bertelli & Mira, 1995). Animals held a grid connected to a force transducer, and then animals were pulled by the tail with increasing firmness until they lose hold. Two days prior to anesthesia, the animals were trained on the equipment. The first evaluation was performed before injection of the anesthetics and the second after 10 minutes of recovery. In each evaluation, the test was repeated three times and used the mean value of replicates.

Evaluation of nociception

To assess the nociception, we used a digital von Frey filament (Insight®). The test was performed with the animal manually restrained and the digital Von Frey filament applied to the right hind limb, both in the plantar region and just below the incision. The tip of the polypropylene filament was applied perpendicularly to the area with gradually increasing pressure, and then the animal withdrew the paw, the test was stopped and recorded withdrawal threshold. Tests were trained two days before the completion of the injury procedure and performed prior to anesthesia 10 and 60 minutes after awakening the animals. At the end, the animals were euthanized by decapitation in guillotine.

Statistical analysis

Data were presented by descriptive statistics (mean and standard deviation). Data normality was checked by Kolmogorov-Smirnov test, and the Student's *t*-test for unpaired values was applied to compare anesthesia time. For the other variables (nociception and grip strength), a mixed model ANOVA was used. In all cases, the level of significance was 5%.

Results

Anesthesia time

The anesthesia time measured for G1A was 70'13" ± 18'05", whereas for G1AL group the time was significantly shorter, 45'07" ± 18'20" ($p = 0.0031$).

Evaluation of grip strength

In the assessment of grip strength, there were ~~no~~ significant differences ($F (1; 21) = 73.0$; $p < 0.001$). There were no differences between groups ($p = 0.459$), but the pre-anesthesia values were significantly higher ($p < 0.001$) (Table 1).

Table 1. Values for grip strength (in grams) in pre- (Pre-) and 10 minutes (Post-) after awakening animals, for only anesthetized (G1A) or anesthetized plus LLLT (G1AL) groups.

	Pre	Post
G1A	705.60 ± 130.20	393.10 ± 116.50
G1AL	654.60 ± 162.30	369.40 ± 162.70

Evaluation of nociception

For plantar region, there were significant differences ($F (1.52; 31.9) = 48.9$, $p < 0.001$), whereas the laser group had withdrawal threshold significantly higher than the control group ($p = 0.019$). Regarding the evaluation periods, the pre-value was significantly greater ($p < 0.001$), but no difference was found between 10 and 60 minutes ($p = 0.335$) (Table 2).

To the pressure performed next to the incision site was also significant ($F (1.38; 29.1) = 52.5$, $p < 0.001$). Again, the G2AL threshold showed greater withdrawal than G2A ($p = 0.032$), and the pre- value was significantly higher ($p < 0.001$), with no differences between 10 and 60 minutes ($p = 1$) (Table 2).

Table 2. Values found for the limb withdrawal threshold (in grams) for the pressure performed on the plantar surface (Plantar) and close to the incision (Local) in the pre-injury (Pre) times, 10 (10') and 60 (60') minutes of awakening.

		Pre	10'	60'
Plantar	G2A	251.60 ± 29.62	101.90 ± 15.26	95.95 ± 34.99
	G2AL	255.00 ± 25.17	185.50 ± 100.50	159.30 ± 90.16
Local	G2A	374.10 ± 35.03	150.90 ± 34.74	149.10 ± 46.82
	G2AL	382.80 ± 32.34	231.00 ± 145.00	248.70 ± 132.20

Discussion

LLLT is a useful tool for the therapeutic field, aiming tissue repair (Bossini et al., 2012; Cury et al., 2013; Hussein, Alfars, Falih, & Hassan, 2011; Vasilenko et al., 2010), anti-inflammatory effects (Chen, Huang, Sharma, & Hamblin, 2011; Marcos et al., 2011) and analgesia (Chow et al., 2007; Chow, Johnson, Lopes-Martins, & Bjordal, 2009; Chow, Armati, Laakso, Bjordal, & Baxter, 2011; Eslamian, Shakouri, Ghojazadeh, Nobari, & Eftekharsadat, 2012; Rayegani et al., 2011; Yan et al., 2011). However, Aras et al. (2010), in a review study, indicate a possible dubious effect of low power laser associated with anesthetics, since it can help or hinder the effects of anesthesia. Ignatov et al. (2005) observed, after irradiating with HeNe laser on snails neurons associated bupivacaine, potassium channel changes, increasing the effect of blocking or decreasing the anesthetic action, according to the use of high or low doses of irradiation.

On the laser capacity to extend or shorten the duration of the anesthetic effect, both by analgesic effects and by increasing local blood supply, respectively, Aras et al. (2010) report that the LLLT has been used to prevent postoperative pain, although some studies indicate positive effects and others report no effect. In this way, these authors emphasize the need for further clinical and experimental research to investigate the effects of LLLT on the duration of anesthesia. Thus, the present study aimed to evaluate the effects of laser 660 nm, with a total dose of 20 Jcm^{-2} on anesthesia with ketamine and xylazine in rats subjected to anesthesia alone or combined with the exposure of the sciatic nerve to minimize the use of common anesthetics in painful postoperative recovery due to surgical procedures.

The effect of LLLT on microcirculation has been documented by several studies, including Plass et al. (2012), who observed that the 680 nm laser caused vasodilation in coronary and fragments of human internal thoracic arteries. Concurrently, Shuvaeva, Gorshkova, Kostylev, & Dvoretsky (2011) irradiated normotensive or hypertensive rats with 473 and 650 nm and observed that, especially for the red wavelength, the laser weakened arterial tone, thereby increasing the constrictor effect of norepinephrine. Pereira, Pinho, Medrado, Andrade, & Reis (2010) irradiated mice with 670 nm, after performing skin lesions, and verified an increased expression of vascular endothelial growth factor (VEGF), increased acute inflammation and vasodilation. Such effect of laser therapy is based on the premise of Aras et al. (2010), which may have been observed with regard to the time of awakening of the animal, whereas for the irradiated group the time was significantly lower than that to the control group.

The grip strength assessment was used by Bertelli and Mira (1995) to analyze the recovery of median nerve injury. In this study, it has been adapted for the analysis of anesthesia recovery. This assessment is simple and reproducible and may indicate differences in the muscular function and in post-anesthesia recovery. As expected there was a significant decrease in muscle grip strength in the post-anesthetic period, but there were no differences between groups, indicating that, about 10 minutes after recovery from anesthesia, the LLLT did not influence the post-anesthesia care for muscle strength.

Nociceptive assessment was performed by the paw withdrawal threshold for pressure with the electronic von Frey filament, which has been shown to be a sensitive, objective and useful method to quantitatively evaluate nociception and the analgesic effect of therapies (Vivancos et al., 2004). This evaluation evidenced the analgesic effect of LLLT, since the withdrawal threshold was higher for this group, regardless of whether the stimulus was plantar or local, in comparison with the control group.

The results of this study supported the use of laser immediately during intraoperative acts. This fact was similar to that reported by Markovic and Todorovic (2006), who irradiated humans anesthetized with lidocaine associated with epinephrine, locally, after 10 minutes of removal of third molar and noted that the pain was less severe in the group receiving 637 nm LLLT with 4 J cm^{-2} than in groups receiving diclofenac or control group.

As limitations of the present study, stand out the lack of assessments of nerve conduction or inflammatory markers (for the second stage), which is suggested to be performed in future studies.

Conclusion

In summary, LLLT caused no significant reduction on the effect of anesthesia, because, although it has produced early awakening, it did not affect muscle strength, and promoted a better analgesic effect than the non-irradiated condition.

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