Antinociceptive Activity of ST36 Acupoint Stimulation by Low-Level Light Therapy (LLLT) in Mice

Vanessa Erthal, Percy Nohama
Rehabilitation Engineering Laboratory/CPGEI/UTFPR
Curitiba, Brazil
e-mail: acupuntura_vane@yahoo.com.br;
percy.nohama@gmail.com

Maria Fernanda de P. Werner, Cristine H. Baggio
Pharmacology department/UFPR
Curitiba, Brazil
Email: mariafernandaw@gmail.com;
crisbaggio@hotmail.com

***Abstract*— Laser acupuncture is a modality of low-level light therapy used as an alternative to needling for the past three decades. The ST36 (Zusanli) acupoint is used to treat inflammatory processes, pain and gastrointestinal disturbs. For this reason, the aim of the present study was to evaluate the antinociceptive effect of Laser acupuncture (830 nm, 3 J/cm2) on ST36 acupoint model of acute nociception. To contribute to this understanding was investigated the involvement of adrenergic and adenosinergic systems in the antinociceptive effect of ST36 acupoint stimulation in mice model of acute nociception induced by glutamate (20 µmol per paw). Our results demonstrate that ST36 laser acupuncture inhibited the glutamate-induced nociceptive behavior in 44%. Moreover, the antinociceptive activity of laser irradiation in the glutamate test was significantly reversed by pre-treatment of yohimbine (0.15 mg/kg, i.p.) and caffeine (3 mg/kg, i.p.) but not by prazosin (0.15 mg/kg, i.p). Collectively, these results demonstrated that ST36 photonic stimulus showed antinociceptive effect in acute model of nociception in mice, and that this effect is mediated by activation of the α2 adrenergic and adenosinergic systems.**

Keywords: Laser acupuncture; antinocicpetion; Zusanli

I. INTRODUCTION

Laser acupuncture is a type of low-level laser therapy (LLLT) and consists of a non-invasive stimulation of the traditional acupuncture points. Its clinical application is becoming widespread being used to treat pain and inflammation, especially with regard to the control of chronic pain states, avoiding the use of analgesics and anti-inflammatory (NSAIDs) [1-2]. This form of phototherapy has been shown to modulate various biological processes and may be used as a supplementary or alternative treatment of several symptoms [3]. The ST36 (Zusanli) acupoint has been used to treat inflammatory processes, pain and gastrointestinal disturbs [4]. Previous pre-clinical data showed that ST36 stimulation with LLLT, during 2, 6 and 10 min, inhibited the nociception induced by formalin in mice [5]. Besides, a clinical study with LLLT set to 830 nm and 30 mW, applied on ST36 and IG4 acupoints in children reduced the migraine [6]. However, there are few evidences about its efficacy and mechanisms of action. For this reason, the aim of the present study was to evaluate the antinociceptive effect of LLLT on ST36 acupoint and its mechanism of action using models of acute nociception in mice. Although the work is in a preliminary phase, several conclusions can be longer designed specially involving the mechanisms of action of LLLT. The paper was divided into four parts: (I) Introduction, in which we present the scientific fundaments involved in the antinociceptive effect of Laser acupuncture and the goal of the experimental study proposed (II) Material and methods performed on this research, as well as the main experimental models involved; (III) Results related to the application of the experimental protocols and discussion about the main results; (IV) Conclusion, where we highlight the most important findings of the developed study.

II. MATERIAL AND METHODS

A. Animals

Experiments were conducted using female Swiss mice (25–35 g), housed at 22 ± 2 °C under a 12/12 h light/dark cycle (lights on at 06:00 h) and with free access to food and water. All experimental protocols were performed after approved by the Committee of Animal Experimentation of the Federal University of Paraná (CEUA/BIO - UFPR, protocol 514) and were carried out in accordance with the ethical guidelines for investigations of experimental pain in conscious animals [7]. The number of animals and intensities of noxious stimuli set were the minimum value necessary to demonstrate the consistent effects of the treatment.

B. LLLT treatment procedures

For the experiments, a low-intensity AsGaAI laser equipment was used. Its main parameters were: wavelenght of 830 nm (in continuous-mode), fluence of 3 J/cm², power of 30 mW, irradiation area reached 6 mm², duration of 6 s on the acupoint. The animals were randomly divided in three groups (n=8): (1) Control group, which was not

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treated; (2) Laser group, which was treated with unilateral ST36 laser acupuncture; and (3) Off group, in which laser device was turned off but holding the probe in contact with ST36 acupoint. ST36 (Zusanli) acupoint is located between the tibia and the fibula, approximately 5 mm lateral to the anterior tubercle of the tibia [8].

C.. Nociception induced by glutamate

The procedure used was similar to that described previously [9]. A volume of 20µl of glutamate (20 µmol per paw) prepared in isotonic saline solution, with pH adjusted to 7.4 by the addition of NaOH, according to Meotti et al. (2006) [10], was injected intraplantarly (i.pl.) in the ventral surface of the right hindpaw. Animals were observed individually for 15 min following glutamate injection. The amount of time they spent licking the injected paw was recorded with a chronometer and considered as indicative of nociception as Fig. 1. Animals were treated with unilateral ST36 laser acupuncture 30 min before glutamate injection.

D. Involvement of α_1-adrenergic system

To investigate the participation of the α_1-adrenergic system in the antinociceptive effect of ST36 laser acupuncture, mice were pre-treated with prazosin (0.15 mg/kg, i.p, a nonselective α_1-adrenergic receptor antagonist) 30 min before application of unilateral ST36 laser acupuncture, phenylephrine (10 mg/kg, i.p., α1-adrenergic receptor agonist) or vehicle (0.1 ml/10 g, i.p.). Another group of mice was pre-treated with vehicle and after 30 min, received unilateral ST36 laser acupuncture, phenylephrine or vehicle, 30 min before the glutamate injection. The nociceptive response was evaluated as previously reported.

E. Involvement of α_2-adrenergic system

To assess the possible participation of the α_2-adrenergic system, mice were pretreated with yohimbine (0.15 mg/kg, i.p, a nonselective α_2-adrenergic receptor antagonist) 30 min before application of unilateral ST36 laser acupuncture, clonidine (0.1 mg/kg, i.p., α_2-adrenergic receptor agonist) or vehicle (0.1 ml/10 g, i.p.). Another group of mice was pre-treated with vehicle and after 30 min, received unilateral ST36 laser acupuncture, clonidine or vehicle, 30 min before the glutamate injection. The nociceptive response was evaluated as previously reported.

F. Involvement of adenosinergic system

To investigate the involvement of adenosinergic system in the antinociception caused by ST36 laser acupuncture, mice were pre-treated with caffeine (3 mg/kg, i.p., a nonselective adenosine receptor antagonist) 30 min before application of unilateral ST36 laser acupuncture or vehicle

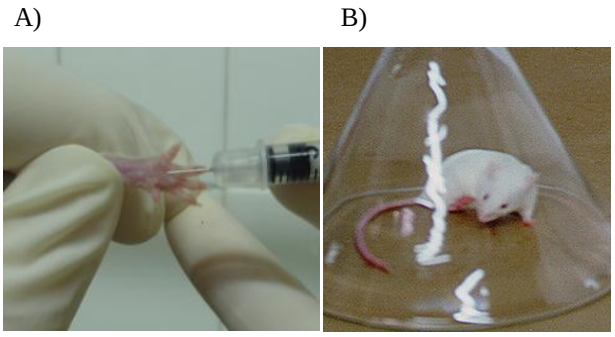

Figure 1. (A) Application intraplantar (ipl.) the glutamate in ventral surface of the right hind paw and (B) observation in the mice glutamate injection.

(0.1 ml/10 g, i.p.). After 30 min, the nociceptive response to the glutamate intraplantar injection was recorded as previously reported.

G. Statistical analyses

Data are presented as means ± standard error of the mean (S.E.M.). Comparisons between experimental and control groups were performed by one-way analysis of variance (ANOVA) followed by Newman Keul's test when appropriate. *P* values less than 0.05 were considered as indicative of significance.

III. RESULTS AND DISCUSSION

The results presented in Fig. 2 demonstrate that ST36 laser acupuncture significantly inhibited nociception induced by glutamate in mice, with inhibition values of 44%. The treatment with laser device turned off was not able to reduce nociception induced by glutamate injection when compared with control group.

In order to identify mechanisms by which it promotes its antinociceptive acupoint, alpha-adrenergic and adenosinergic systems were tested using pre-treatments antagonists of such systems, to evaluate whether some of them would modified the results found in the model of nociception induced by glutamate.

Involved with antinociception, α-adrenergic pathways descendants are recruited from stimuli in brain structures such as Periaqueductal Gray substance (PAG) [11]. The antinociceptive properties of agonists of α2-adrenergic receptors modulation reflect the influence of excitatory neurons in primary afferents projection [10]. Evidence also suggests that α1-adrenergic receptors modulate nociceptive processing in the dorsal horn of the spinal cord [11].

The involvement of adrenergic system on the antinociceptive action of Laser was demonstrated through the pre-treatment of animals with the non-selective adrenergic receptor.

Next, the involvement of the α1 and α2 adrenergic systems on ST36 laser acupuncture was investigated.

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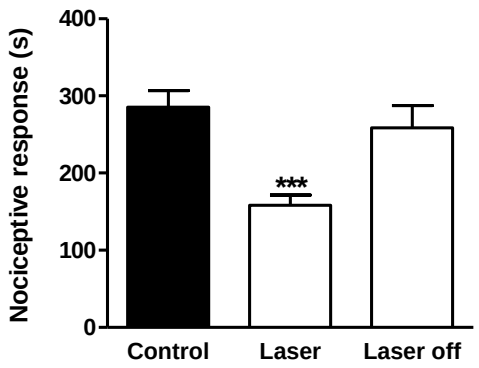

Figure 2. Effect of ST36 laser acupuncture on glutamate induced nociceptive behavior in mice. Each group represents the mean of 8 animals, and the vertical lines indicate the S.E.M. ***p<0.001 when comparing with control group (one-way ANOVA followed by Newman-Keuls Multiple Comparison Test).

Results presented in Fig. 3A show that prazosin pre-treatment completely reverted the antinociceptive effect of both phenylefrina and the effect antinociceptive of ST36 laser irradiation was not reverted that prazosin group in the glutamate test.

Although the results in Fig. 3B show the effect antinociceptive of ST36 laser irradiation was reverted that Yomhibine. Using bee venom injection in acupoint CV12 (Zhongwan), Kwon and colleagues (2001) [12] demonstrated the involvement of this receptor α2-adrenergic antinociception. Park et. al. (2012) [13] observed that α2-adrenoceptor antagonist and β-adrenoceptor antagonist inhibited the analgesic effect of eletroacupuncture (EA) on the inflammatory pain in a rat model of collagen-induced arthritis, but α1-adrenoceptor did not inhibit the analgesic effect of EA, as in our results [13].

Despite being an ancient technique in the east, acupuncture is still a relatively recent scientific study in the west. In fact, there are few studies showing the mechanisms of action and the difference among acupoints in the scientific literature. Another important factor to evaluate is the technique used, since most of the researches employs electroacupuncture, while few of them use photonic stimulation (known as laserpuncture).

Laser light is a good alternative to metal needles for stimulation of acupuncture points, and it has been used successfully for several decades. However, there are only few studies proving the effectiveness of this kind of acupuncture. Most publications focus on red or infrared laser stimulation, and there are several relevant studies [14–17].

The systemic pre-treatment of mice with caffeine in Fig. 4 did significantly reverse the antinociception caused by Laser and against glutamate induced nociception. The findings of Goldman et al. (2010) [18] indicate that adenosine is central to the mechanical actions of acupuncture, as the results demonstrated in our experiments in laser acupuncture.

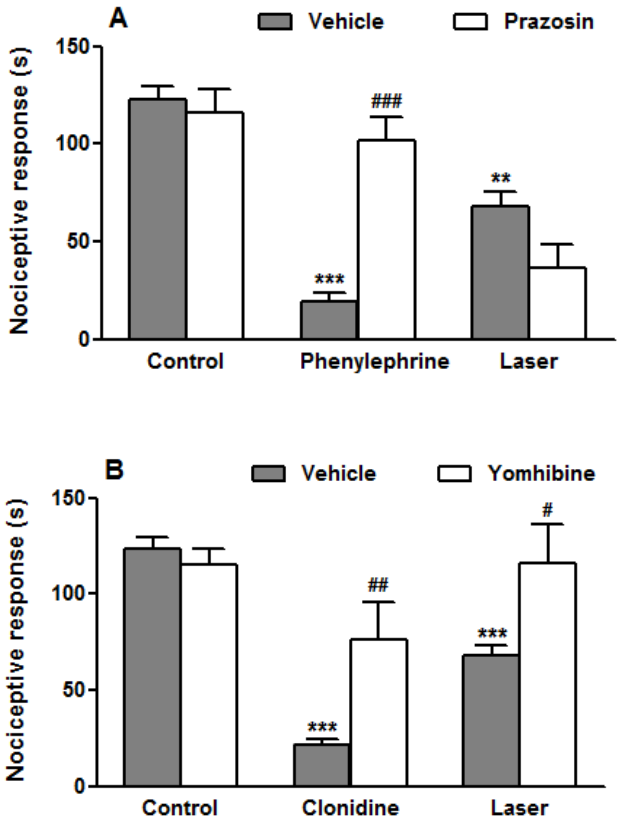

Figure 3. Effect of pre-treatment with prazosin (Panel A), yohimbine (Panel B) on the antinociceptive effect of ST36 laser acupuncture, phenylephrine, vehicle or clonidine. Each group represents the mean of 8 animals, and the vertical lines indicate the S.E.M.in the ***p<0.001 when comparing to control group; $^{\#\#}$p<0.01 and $^{\#}$p<0.05 when comparing antagonist vs antagonist + agonist or laser treatment. (one-way ANOVA followed by Newman-Keuls Multiple Comparison Test).

IV. CONCLUSION

LLLT, one of the most recent and promising treatment therapies in the acupuncture, has been shown to reduce to relieve pain significantly. In summary, the present study demonstrated that stimulation of the acupoint E36 shows antinociceptive activity in nociception model with glutamate and that this action seems to be involved in the activation of α2 adrenergic and adenosinergic systems.

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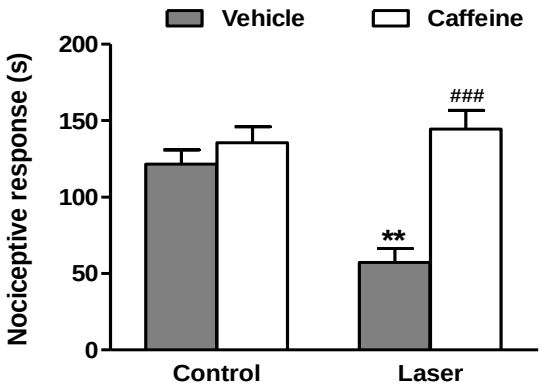

Figure 3. Effect caffeine pre-treatment on the antinociceptive effect of ST36 laser acupuncture in the glutamate test. Each group represents the mean of 8 animals, and the vertical lines indicate the S.E.M. **$p<0.01$ when comparing to control group; ###$p<0.001$ when comparing treatment vs caffeine+ treatment (one-way ANOVA followed by Newman-Keuls Multiple Comparison Test).

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