

The Anti-inflammatory Effects of Laser Acupuncture at ST36 (Zusanli) Acupoint in the Model of Acute Inflammation Induced by Carrageenan in the Paw of Mice

Vanessa Erthal, Percy Nohama*

Rehabilitation Engineering Laboratory/CPGEI
Federal Technological University of Paraná UTFPR
Curitiba, Brazil
e-mail: acupuntura_vane@yahoo.com.br

Maria Fernanda de P. Werner, Cristiane H. Baggio

Pharmacology Department
Federal University of Paraná UFPR
Curitiba, Brazil
e-mail: crisbaggio@gmail.com

Abstract— Low-intensity Laser acupuncture (LA) has been applied as an alternative to needling along the past three decades. The ST36 (Zusanli) acupoint is used to treat inflammatory processes, acute pain and gastrointestinal disturbs. For this reason, the aim of the present study was to evaluate the anti-inflammatory effect of Laser acupuncture (830 nm, 4 J/cm²) on ST36 acupoint on paw edema induced by carrageenan in mice, a model of acute inflammation. Mice were treated with LA 30 min before intraplantar injection of carrageenan (300 µg/paw). The formation of edema was assessed using a digital micrometer and temperature analyzed through thermography. The results indicate that ST36 LA significantly inhibited the paw edema induced by carrageenan and reduced the temperature on skin plantar surface. In conclusion, these results demonstrated that ST36 photonic stimuli have anti-inflammatory effect in acute model of inflammation in mice.

Keywords: Laser acupuncture; inflammation; edema.

I. INTRODUCTION

Acupuncture is an effective procedure for pain relief, nausea and vomiting, bronchial asthma, musculoskeletal disorders and inflammatory conditions [1]. There are different acupuncture techniques, which traditionally use needle puncture [2]. However, laser acupuncture (LA) is a noninvasive and noninfectious method that can avoid pain and psychological fear promoted by the insertion of needles [2-5]. Indeed, LA is a form of phototherapy at acupoint similar to needle acupuncture, differing in the type of stimulus [6].

The ST36 (Zusanli) acupoint has been used to treat inflammation, acute pain and gastrointestinal disturbs [7]. Previous studies showed that ST36 acupoint stimulated with Low Level Laser Therapy (LLLT), during 2, 6 and 10 min, inhibited the nociceptive response induced by formalin in mice [8]. Recently, Erthal et al. [4] also demonstrated that ST36 stimulation with Gallium Aluminium Arsenide (GaAlAs) laser elicited significant antinociceptive effect against acetic acid- and formalin-induced nociception in rats, with participation of opioidergic and serotonergic systems. Moreover, it has been reported that the application of ultra low level laser therapy (ULLLT) on ST36 and TB5

acupoints also reduced acute and chronic inflammation induced by carrageenan and complete Freund's adjuvant, respectively (for review see Baratto et al. [9]). Interestingly, a clinical study with LA, set to 830 nm and 30 mW, applied on ST36 and IG4 acupoints reduced significantly the migraine in children [10].

Carrageenan is a substance widely used for induction of inflammation in animal models, and is a test employed to assess the effects of alternative methods, as LA, for inflammation and pain control [11]. It is well known that temperature can be a parameter in models of inflammation and several studies with acupuncture use the local measuring of temperature to evaluate its effect on inflammatory processes. Sanchez et al. [12] demonstrated that the thermal imaging technology (thermography) is a rapid, highly reproducible method to quantify the degree of inflammation in rat models of general inflammation.

However, further studies must be done to deeply assess its clinical efficacy and to investigate the molecular mechanisms involved in its effect. Despite the stimulation of ST36 acupoint being used to treat inflammatory conditions, there are few evidences demonstrating the effectiveness of LA for reducing edema due to inflammation. For this reason, the aim of the present study was to evaluate the anti-inflammatory effect of LA on ST36 acupoint using the acute inflammatory model in mice.

The paper was divided into four parts: (I) Introduction, in which we present the scientific fundamentals involved in anti-inflammatory 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; and (IV) Conclusion, where we highlight findings on the performed 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 they were approved by the Committee of Animal Experimentation of the Federal University of Paraná (CEUA - UFPR, protocol number 514).

B. LLLT treatment procedures and body location

For the experiments, a low-intensity GaAlAs laser equipment was used. Its main parameters were: wavelength of 830 nm (in continuous-mode), fluence of 4 J/cm², power of 30 mW, irradiation area reached 6 mm², duration of 8 s on the acupoint. The animals were randomly divided into four groups (n=8 animals per group): (1) Control group, which was not treated; (2) Laser on group, which was treated with unilateral ST36 laser acupuncture; and (3) Laser off group, in which laser device was turned off but holding the probe in contact with ST36 acupoint; (4) Dexamethasone group [DEXA, 0.5 mg/kg, intraperitoneal (i.p.)], a positive control of the test. ST36 (Zusanli) acupoint is located between the tibia and the fibula, approximately 5 mm lateral to the anterior tubercle of the tibia [4].

C. Acute inflammation induced by carrageenan

The animals were treated with laser and DEXA and, after 30 min, an intraplantar (i.pl.) injection of carrageenan (300 µg/paw, 20 µl) was administered into the right hind paw of the mice. The thickness of the paw was measured using a digital micrometer (Great, MT-045B) before the induction of edema (B: basal) and at different time points after the injection of the phlogistic agent. All of the assessments were performed by the same investigator in order to reduce any potential inter-operator differences.

D. Thermographic analysis

Temperature measurements of the hind paw's plantar surface were obtained by an Infrared Camera, model A325 (FLIR Systems, Inc.). The main parameters of the thermographic camera are: acquisition frequency of 60 Hz with 16 bits-resolution, 320 x 240 pixels image resolution, detecting wavelengths from 7.5 up to 13 µm, lens incorporating autofocus, temperature measurement in the range of -20 to +120 °C, with 2% accuracy, thermal resolution of 0.08 °C and 0.1 mm of spatial resolution. The software used for thermographic images acquisition, storage and analysis was the ThermaCAM Researcher Pro 2.9, developed by FLIR Systems, Inc. Skin temperature was measured after leaving each animal at least one hour to acclimate with the laboratory temperature. Animals were lightly anesthetized with sodium pentobarbital (30-40 mg/kg, i.p., Cristália, Brazil) to suppress the righting reflex while preserving the withdrawal reflex. Anesthetized mice were gently placed on the box, and the dorsal surface of the hind paw was fixed. The hind paws were positioned, and the

heat emitted from the plantar region was measured using the infrared camera.

E. Statistical analysis

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

III. RESULTS AND DISCUSSION

Inflammation is the body's immediate response to the tissue damage and defined by vasodilation, exudation of fluid and cell migration. In 1962, the carrageenan-induced inflammatory response was described for the rat paw, and in 1969 for mice [13]. Since that time, edema on mice's paw has been increasingly used to test new anti-inflammatory drugs and treatments [14]. Then, we used this model of acute inflammation induced by carrageenan to evaluate the anti-inflammatory effect of LA treatment. In our experiments, it was observed a rapid onset of paw edema in the control group after i.pl. injection of carrageenan. Interestingly, the results depicted in Fig. 1 indicate that ST36 LA significantly inhibited the paw edema at 2 and 3 h after phlogistic agent injection, with inhibitions of 13 and 18%, respectively. However, the treatment with laser device turned off was not able to reduce the edema when compared to the control group. DEXA, a steroidal anti-inflammatory drug and positive control of the test, also reduced the paw edema at 2 and 3 h (Fig. 1). It is known that laser therapy activates both local microcirculation and cellular metabolism, and produces anti-inflammatory, analgesic and regenerative effects [15], suggesting that these factors could be involved in our LA treatment. In accordance with our findings, another type of LA, the ULLLT, is also able to inhibit the paw edema induced by carrageenan [16]. Lee et al. [17] showed that electroacupuncture, applied on ST36 and SP6 acupoint, on different frequencies such as 2, 15 and 120 Hz, produced relevant anti-edema effects compared with control group. For laser therapy, a range of wavelengths 633.8 up to 904 nm can be applied [18]. Besides, according to the Arndt-Schultz law for biostimulation, anti-inflammatory and analgesic effects occur at doses between 0.05 and 10 J/cm² [19]. In this study, LA with wavelength of 830 nm and radiant exposure of 4 J/cm² showed significant effects on the inflammatory model. In previous experimental studies in our laboratory, the applied dose of 3 J/cm² demonstrated best responses on inflammatory and nociception models. In studies applying electroacupuncture on ST36 point, Park and colleagues [20]

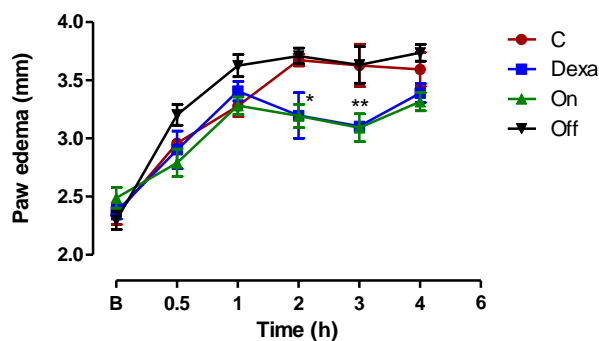


Figure 1. Effects of LA or DEXA on carrageenan-induced edema. Edema of the carrageenan-inflamed hindpaw was determined by measuring paw thickness with micrometer (n=8), indicate the S.E.M. **p<0.01.

reported effectiveness of this acupoint for anti-inflammatory and anti-arthritis on collagen-induced arthritis (CIA), via suppressing autoimmunity and modulating immune abnormality. Kim et al. demonstrated that EA stimulation of the Zusanli acupoints produced significant suppressive effect on carrageenan-induced paw inflammation and hyperalgesia [21]. Thermography has been useful for diagnosis of inflammatory processes because it can assess variations in skin surface temperature. It is highly sensitive and noninvasive, capable of detecting very small alterations on skin temperature [22]. The thermal image analysis involves measurements of the surface temperature the body using an array of infrared sensors installed inside the camera. This image allows the simultaneous measurement of temperatures of multiple points on the skin [23]. The images and the graphic illustrated in Fig. 2 show the skin temperature change for the plantar surface of the hind paw. Changes were measured through the infrared camera adjusted to the range of 25 to 37 °C. Fig. 2c shows that the inflammation promoted by carrageenan increased the temperature of plantar surface in 14% (Naive: 26.5 ± 0.5 °C). However, the treatment of animals with LA on acupoint ST36 reduced the temperature in 12% when compared with control group (Control: 30.1 ± 1.0 °C). By means of the thermographic analysis, we can conclude that LA on the ST36 acupoint has an anti-inflammatory action.

IV. CONCLUSION

Several years of research have produced a steady stream of laser acupuncture studies; however, the objective assessment and reproducibility of results are difficult because of the lack of information about the main physical parameters set. However, recent studies have shown that laser therapy when administered by a specific emission mode may elicit significant biological effects

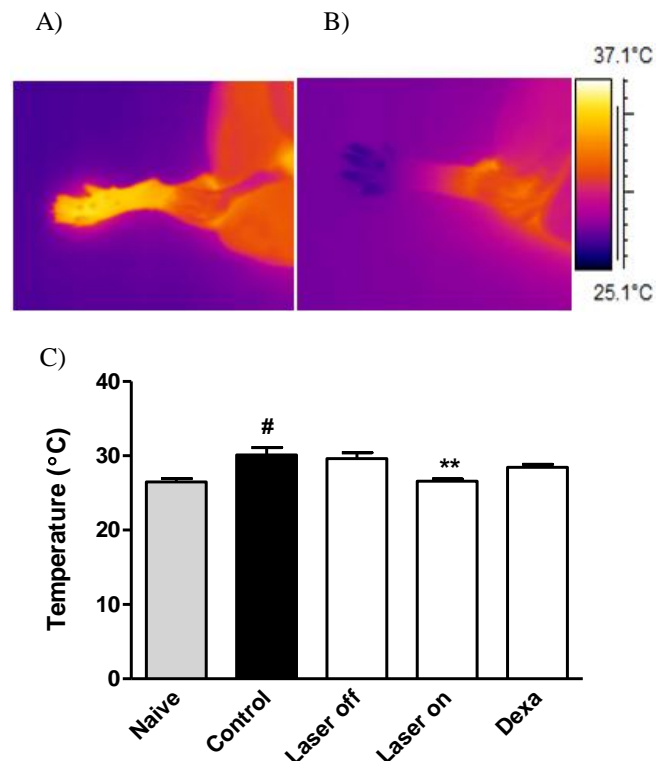


Figure 2. Digitized images of hind paw plantar surface skin temperature, the control (A) and laser on (B) groups. (C) Effect of ST36 laser acupuncture or DEXA in carrageenan-induced paw in mice. Each group represents the mean of 8 animals, and the vertical lines indicate the S.E.M. **p<0.01 and #p<0.05 when comparing with control group.

Finally, this study allows us to conclude that stimulation of the acupoint ST36 with LLLT produces relevant suppression of carrageenan-induced paw edema. Further studies have been and will be carried out in our laboratory to understand the effects of LA on inflammation.

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