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HEALTH SCIENCES

Acupuncture and laserpuncture as a therapeutic approach for nociception and inflammation: An experimental study in mice

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Abstract: Inflammation and pain are consequences of injuries or diseases that affect a large number of people. This study aims to evaluate the effect of acupuncture and laserpuncture on nociception and inflammation in mice compared to the effects of morphine and dexamethasone. 140 male Swiss mice were used. Treatment with acupuncture and laserpuncture were performed at the acupoints LI11, ST36, GB34, and BL60 in mice. To evaluate the effect of acupuncture and laserpuncture on nociception, the hot plate test and intraplantar formalin injection were used. The effect of acupuncture and laserpuncture on the inflammation was evaluated through carrageenan-induced paw edema. Thermographic analysis was also applied to evaluate the anti-inflammatory effects. An antinociceptive effect (≈57%) was observed in treatments with acupuncture and laserpuncture, equivalent to the effect of morphine. Laserpuncture and acupuncture decreased paw edema by ≈25%. Acupuncture had an effect equivalent to dexamethason, basides reducing the neurogenic phase by 35% and the inflammatory phase in formalin-induced nociception by 40%, equivalent to the effects of morphine. In thermographic analysis, acupuncture, laserpuncture, morphine, and negative control had paw temperature of ≈27 °C, while formalin treatment was 31°C. Acupuncture and laserpuncture proved to be effective therapies for the treatment of inflammatory and painful processes.

Key words: acupuncture, inflammation, laserpuncture, mice, pain.

INTRODUCTION

Inflammation is a reaction in response to the invasion of an aggressive agent and its main function is to protect the body against infections and repair tissues after injuries. There are five basic signs of the inflammatory response: redness, edema, heat, pain, and loss of function. These signs occur due to an influx of inflammatory cells, arterial enlargement, increased blood flow, extravasation of plasma proteins, and edema formation (Chen et al. 2018).

The word pain is defined, according to the International Association for the Study of Pain (IASP), as "an unpleasant sensory and emotional experience associated with, or similar to that associated with, actual or potential tissue damage" (Raja et al. 2020). It is a pathophysiological consequence of several morbidities, configuring itself as a protective function and, in many cases, it is the only symptom for the diagnosis of several diseases (Oliveira et al. 2009).

As animals do not verbally express the subjective components of pain, it is expressed

in nociception. Nociception refers to the painful stimulus itself, without considering the emotional component. It encompasses the neuroanatomical pathways, the neurological mechanisms, and the specific receptors that detect the injurious stimulus (Kandel et al. 2003).

Drugs such as opioids, non-steroidal anti-inflammatory drugs, local anesthetics, α-2 agonists, and dissociative anesthetics are administered to control pain and inflammation in animals (Romeu et al. 2019). Morphine is an opioid drug, traditional and more effective, used to control pain in small animals (Romeu et al. 2019). Dexamethasone with similar effects to the ones of endogenous cortisol, with anti-inflammatory and immunosuppressive effects, widely used in medical practice. It is recommended for several diseases (Gilroy et al. 2004).

Despite all the progress achieved by medicine, it is known that drugs can promote adverse reactions (Gilroy et al. 2004). Thus, complementary methods are being increasingly used for the treatment of pain and inflammation. Among the recognized non-pharmacological techniques, there is acupuncture, which activates the sensitive-discriminatory system, stimulating the pain suppressor system (Santos & Martelete 2004).

The word acupuncture refers to the insertion of needles through the skin at specific points (acupoints) located on a system called meridians. It aims to produce a therapeutic effect (Schwartz 2008). Acupuncture points can also be stimulated through laserpuncture. Low-level laser therapy has a physiological action that depends on a series of specific properties of its electromagnetic nature. The mechanisms responsible for the analgesic and anti-inflammatory effects are due to the increase in peripheral neurochemical modulations (Pryor & Millis 2015).

Acupuncture research is important to elucidate the phenomena associated with its mechanism of action and to investigate its importance in pain and inflammation control therapy. As it is a low-cost, economical, and practically side-effect-free approach, it could become a first-line resource in pain management. To demonstrate its effectiveness, it is necessary to study it from the western point of view, using classical scientific methodology (Teixeira 2010).

Thus, this study aims to evaluate the effect of acupuncture and laserpuncture on nociception and inflammation in mice compared to the effects of morphine and dexamethasone.

MATERIALS AND METHODS

Animals

To carry out the experiments, 140 male Swiss mice of the Mus musculus species (30-40 g) were used, authorized by the Ethics Committee in the Use of Animals (CEUA) of the Universidade Regional de Blumenau, under protocol No 016/2017. The animals were conditioned at 22 ºC with a 12 hours light/12 hours dark cycle and water and feed *ad libitum*. Each experimental group is composed of 8-10 animals. No animal was submitted to more than one test.

Treatments

For acupuncture and laserpuncture procedures, the right side of the animal was chosen and always used the sequence LI11, ST36, GB34, and BL60 (figure 1). These points were mapped in mice (Li et al. 2004, Yin et al. 2008). The ST36 (Suzanli), BL60 (Kunlun), LI11 (Quchi), and GB34 (Yanglingquan) acupoints were chosen based on the effects observed in humans: ST36 - analgesic, anti-inflammatory, and immunity booster; BL60 – analgesic and myorelaxant; LI11 - increased immunity, analgesic, and

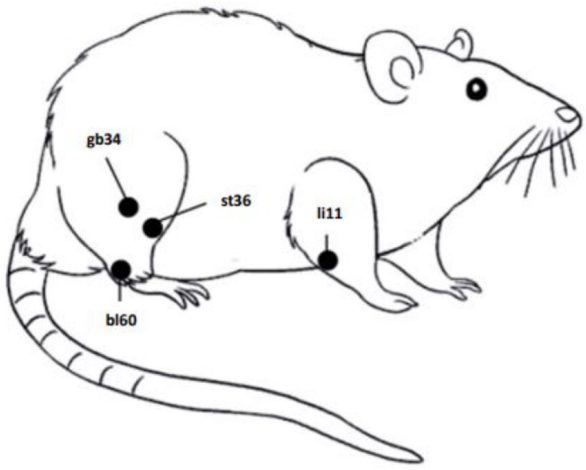

Figure 1. Anatomical location of acupoints bl60, li11, st36, and gb34 in *Mus musculus* mice used in the acupuncture and laser therapy.

anti-inflammatory; VB 34 – myorelaxant, analgesic, and anti-inflammatory.

For the acupuncture procedure, the animals had the needles (0.18x8.0 mm - Korean Dong Bang) inserted for 10 minutes in the body (Silva 2013), under observation, without any type of restraint, free in a 100 x 30cm glass funnel, to avoid stress.

Laserpuncture was performed using a low-intensity laser (TF Premier Plus by MMO) with a wavelength of 808 nm in the infrared range of a semiconductor diode (gallium-aluminum-arsenide) direct current (CW) and the energy density of 5 J/cm², power of 20 mW, irradiation area of 6 mm² and duration of 10 seconds, with beam output with an active tip of the laser pen of 0.0078 cm2, class 3b (MM Optics Ltda, Brazil). The laser was used comfortably on the skin, with the acupuncture tip inclined at 45º. The applicator wore goggles.

The drugs and concentrations used were morphine 1 mg/kg and 5 mg/kg, s.c. (DImorf®, morphine sulfate, Cristália, São Paulo, Brazil), dexamethasone 0.5 mg/kg, s.c. (Decadron®, EMS, São Paulo, Brazil) carrageenan 100 mg/paw (i.pl.) (Sigma Aldrich, Germany), and 2.5% formalin (i.pl.) (Formaldehyde, Commercial Neon Ltda, Suzano, São Paulo, Brazil). The drugs were diluted in isotonic saline solution (NaCl 0.9%).

Hot plate induced thermal nociception

To induce thermal nociception, the hot plate test was used. The animals were randomly divided into six groups:

- Control (n=10) - Animals received isotonic saline solution s.c.
- Morphine (n=10) - Animals received 1 mg/Kg s.c. of morphine.
- Morphine (n=10) - Animals received 5 mg/kg s.c. of morphine.
- Acupuncture (n=10) - Animals were submitted to the acupuncture protocol using the points: LI11, ST36, GB34, and BL60.
- Acupuncture (n=10) - Animals were submitted to the acupuncture protocol using the point BL60.
- Laser (n=10) - Animals were submitted to the laserpuncture protocol using the points: LI11, ST36, GB34, and BL60.

All groups underwent the following treatment before the hot plate test: laser for 30 minutes and acupuncture for 10 minutes afterwards at acupoints LI11, ST36, GB34, and BL60, acupuncture for 10 minutes before at acupoint BL60, morphine (1 and 5 mg/kg) and control (isotonic saline solution) 30 minutes before.

The nociceptive behavior was induced using the hot plate method (Nucci et al. 2012). After treatment, the animals were placed in the hot plate apparatus (50±1ºC) in a 24 cm diameter glass cylinder on the heated surface. The time elapsed between placing the animal on the heated surface and the emergence of rocking, biting, or licking the hind paw on the hot plate was recorded and considered as a latency index. A maximum time of 60 seconds was used to prevent possible tissue damage.

Carrageenan-induced paw edema

To assess paw edema, the animals were randomly divided into four groups:

- Control (n=10) - Animals received only carrageenan 0.1 ml i.pl. (right paw).
- Dexamethasone (n=10) - Animals were treated with 0.5 mg/kg s.c of Dexamethasone.
- Acupuncture (n=10) - Animals were treated with the acupuncture protocol using the points: LI11, ST36, GB34, and BL60.
- Laser (n=10) - Animals were treated with the laserpuncture protocol using the points: LI11, ST36, GB34, and BL60.

All groups underwent treatment before the intraplantar injection (i.pl.) in the right paw with 0.1 ml carrageenan (Tratsk et al. 1997). The animals were treated with acupuncture for 10 minutes, laser for 30 minutes and dexamethasone 30 minutes before intraplantar injection carrageenan. The control group had only carrageenan. The edema was measured using a 799A series electronic digital caliper (Starrett®), in paw swelling micrometers, at time intervals of 30, 60, 120, and 240 minutes after carrageenan injection.

Formalin-induced nociception

To evaluate the formalin nociception test, an intraplantar injection of 20 µl of formalin was performed in the ventral region of the right hind paw of each animal (Hunskaar et al. 1985). For this test, the animals were divided into four groups:

- Control negative (saline solution) (n=10) - Animals received saline solution.
- Control (n=10) - Animals received 20 µl of formalin.
- Morphine (n=10) - Animals were treated with 1 mg/Kg s.c. of morphine.
- Acupuncture (n=10) - Animals were treated with the acupuncture protocol using the points: LI11, ST36, GB34, and BL60.
- Laser (n=10) - Animals were treated with the laserpuncture protocol using the points: LI11, ST36, GB34, and BL60.

All animals underwent treatment before intraplantar injection with formalin: acupuncture for 10 minutes, laser for 30 minutes before at acupoints LI11, ST36, GB34, BL60, and morphine (1 mg/kg) 30 minutes before the test with formalin. The control group had only formalin.

This model allows us to show two phases of nociception: the first (F1 - neurogenic) occurs during the first 5 minutes after the formalin injection, and the second (F2 - inflammatory) occurs between 15 and 30 minutes after the injection. The animals received 20 µl of formalin. After the formalin injection, the animals were placed one by one in a glass funnel, next to a mirror to help observation. The time that the animal remained licking or biting the paw in each experimental group was timed, and this time was considered indicative of nociception.

Thermographic analysis

The groups of animals used in the nociception test induced by the intraplantar injection of formalin were submitted to thermographic analysis, which checks the production of heat through skin temperature to evaluate the anti-inflammatory activity. The experiment was carried out on the plantar surface of the paw of the mice after the treatments.

To measure the temperature, an infrared camera model C2, from FLIR Systems, Inc (USA) was used. This camera operates at an acquisition frequency of 60Hz, with a resolution of 320x240 pixels, in 16 bits, and detects radiation in the spectral range of 7.5 to 13 µm. The lens incorporates monitored focus and autofocus, temperature measurement in the range of -20

ºC to +120 ºC, with 2% error, thermal resolution of 0.08 ºC, and spatial resolution of 0.1 mm. The sensor converts the infrared energy radiated from the skin into a thermogram that represents the exact temperature of its surface. The software used for the acquisition, storage, and analysis of thermographic images was ThermaCam Researcher Pro 2.9, from FLIR Systems, Inc (USA). After acquiring the images, an area in a circle was selected for analysis of the average temperature of the chosen location. Temperature decreases indicate an anti-inflammatory effect. The temperature represents the average of the group, containing from 8 to 10 animals.

Statistical analysis

Data were verified in relation to their normal distribution, according to the Shapiro-Wilk test ($p<0.05$). The results were expressed as mean ± standard error of the mean. The results were submitted to ANOVA, One-Way or Two-Way analysis of variance, with Student-Newman-Keuls post-hoc. Temperature results from thermographic analysis were submitted to ANOVA, One-Way analysis of variance, with Tukey Test post-hoc. All analyses were performed using the GraphPad Prism software version 5.01 (2005, San Diego, CA, USA). Significance was defined as $p<0.05$.

RESULTS

The results for the hot plate test showed that there was an increase of approximately 57% in paw withdrawal latency in the acupuncture and laserpuncture groups, equivalent to that observed in the morphine 1 mg/kg group when compared to the control. Such an increase is also observed when stimulating the BL60 acupoint alone. This point was tested individually in this experiment, as in Traditional Chinese Medicine it is a point responsible for reducing heat. So, in this case, we wanted to show the effect of this point specifically on this mechanism of action. The other experiments were not performed with this individual point. Morphine 5 mg/kg had the longest paw withdrawal latency time when compared to all groups (figure 2).

In the paw edema test, induced by carrageenan, the laserpuncture promoted a decrease in edema by around 14% in 30 minutes, 11% in 60 minutes, and 24% in 240 minutes compared to the control. Acupuncture also demonstrated effectiveness when compared to the control. It decreased the edema by around 12% for the times 30, 60, and 120 minutes, and approximately 27% in 240 minutes. The effects shown at 30 minutes were the same between the laserpuncture, acupuncture, and dexamethasone groups, and at 60 minutes the effects of acupuncture and laserpuncture were also equivalent, while the effects of dexamethasone were slightly greater, at approximately 17%. At 120 minutes, positive effects were only observed for the groups

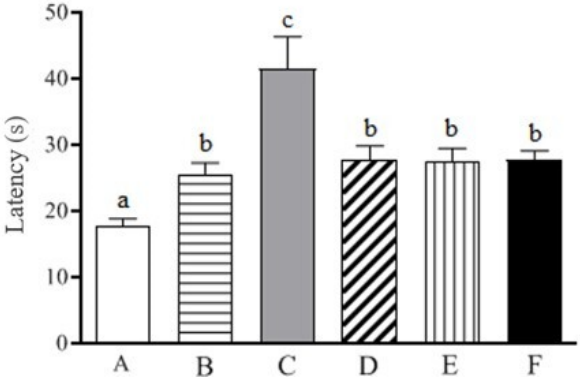

Figure 2. Latency time (s) of paw withdrawal response in mice treated with morphine, laser therapy, and acupuncture over hot plate-induced thermal nociception. Data were expressed as the mean ± SEM from 8 to 10 animals in each group. Different letters indicate statistical differences between treatments ($p<0.05$). (A) Control (B) Morphine 1 mg/kg s.c. (C) Morphine 5 mg/kg s.c. (D) Laser therapy at acupoints bl60, li11, st36, and gb34. (E) Acupuncture at acupoints bl60, li11, st36, and gb34. (F) Acupuncture at point bl60.

treated with acupuncture and dexamethasone, with dexamethasone being more efficient. The 240-minute analysis showed a decrease in paw edema for the laserpuncture, acupuncture, and dexamethasone groups, being the same for the acupuncture and dexamethasone groups, and the same between acupuncture and laserpuncture, showing the effectiveness of laserpuncture and acupuncture in reducing edema (figure 3).

The results shown in figure 4 (1 and 2) show that the previous stimulation of acupuncture reduced by 35% the neurogenic phase (0-5 minutes) (figure 4-1) and 40% the inflammatory phase (15-30 minutes) (figure 4-2) of formalin-induced nociception when compared to the control. These results were similar to the effects of Morphine at 1 mg/kg.

Figure 5 illustrates the variation in skin temperature on the plantar surface of the right hind paw of the mice. The animals in the positive control group, which received intraplantar formalin (figure 5a) showed an increase in temperature, with a mean of 31 °C, when compared to the negative control, with a mean of 26 °C (figure 5Sal). Figure 5b (Morphine 1 mg/Kg), figure 5d (laserpuncture), and figure 5e (acupuncture) showed a decrease in temperature, with an average of approximately 27 ºC, a value close to the negative control. The graph (Figure 5f) represents the mean of all experimental groups.

DISCUSSION

Acupuncture acts on pain control by activating opioid and non-opioid pathways. The stimulation promoted by this technique activates the pain modulating system by hyper stimulating the nerve endings of A-δ myelin fibers, responsible for driving the stimulus to the spinal and brain centers and the hypothalamic-pituitary axis. In

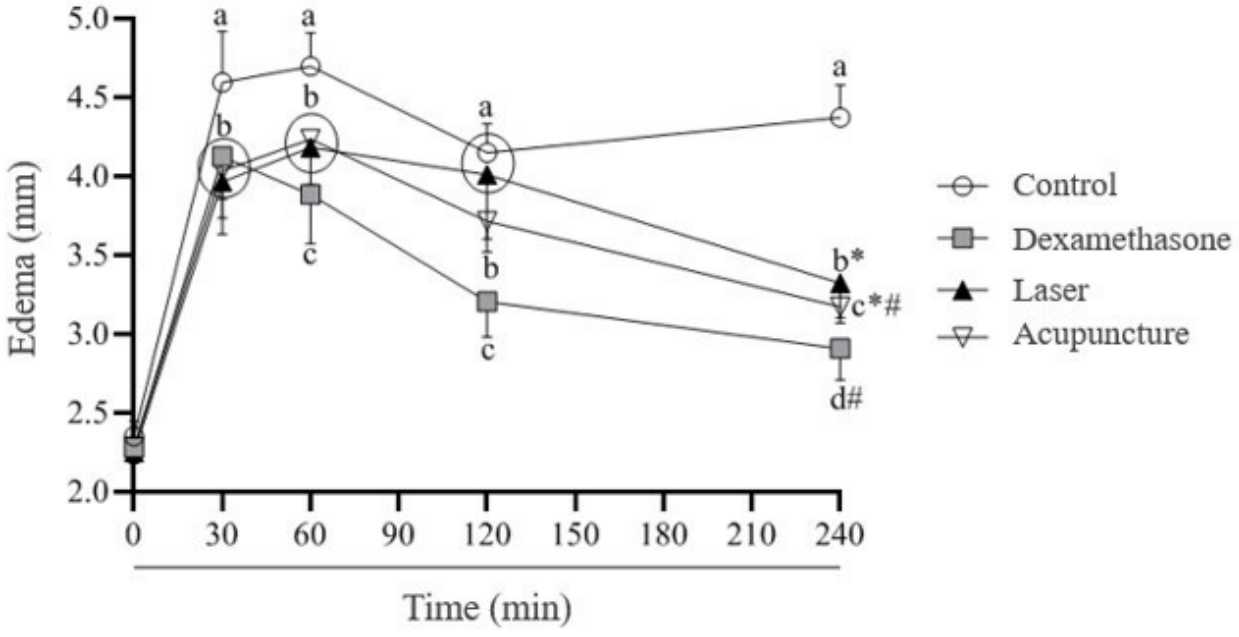

Figure 3. **Paw edema (mm) in mice treated with dexamethasone (0.5 mg/Kg), laser therapy, and acupuncture, in acupoints bl60, li11, st36, and gb34 at different time intervals after the intraplantar injection of carrageenan. Each group represents the average of 8 to 10 animals and the vertical lines represent the SEM. Different letters (a, b, c, and d) and symbols (*, and #) indicate a statistical difference between treatments (p<0.05). Equal letters and symbols showed no statistical difference.**

the spinal cord, the modulation of nociceptive stimuli occurs by presynaptic inhibition due to the release of enkephalins and dynorphins. In the midbrain, enkephalins and the activation of the central pain modulation system result in the release of serotonin and norepinephrine in descending systems (Lin & Chen 2008).

The analgesic effect of electroacupuncture is mediated by μ receptors, resulting in the release of endomorphin/endorphin and δ receptors, releasing enkephalin (Lin & Chen 2008), since the continuous stimulation of acupuncture points for 30 minutes is necessary for the endogenous release of opioids and consequently a possible analgesic response (Luna 2002). Goldman et al. (2010) also showed in their results that the greatest antinociceptive effect was 30 minutes after acupuncture. This time corroborates what was used in this work.

Regarding drugs, morphine is the prototype of opioids or analgesics of central action, which has a high affinity with receptors of the opioid system, in which a small fraction of its dose is sufficient to cross the blood-brain barrier and enter the central nervous system to produce analgesia (Rang et al. 1997). In addition, according to some studies, morphine can also act in the periphery, attenuating hyperalgesia in inflammation models. This effect may be associated with a decrease in the excitability of nociceptors present in inflamed tissues. A reduction in inflammatory cytokines and paw edema resulting from morphine treatment has also been observed in such models (Amann et al. 2002, Wenk et al. 2006).

According to the above, in this study, the anti-inflammatory and analgesic effect of the drug morphine 5 mg/kg prevailed due to the high dosage and its pharmacokinetics, but in relation to morphine 1 mg/kg, the response was similar to stimulation with acupuncture and laserpuncture in the acupoints ST36, BL60, LI11, and GB34, showing that these alternative therapies may also be responsible for inducing

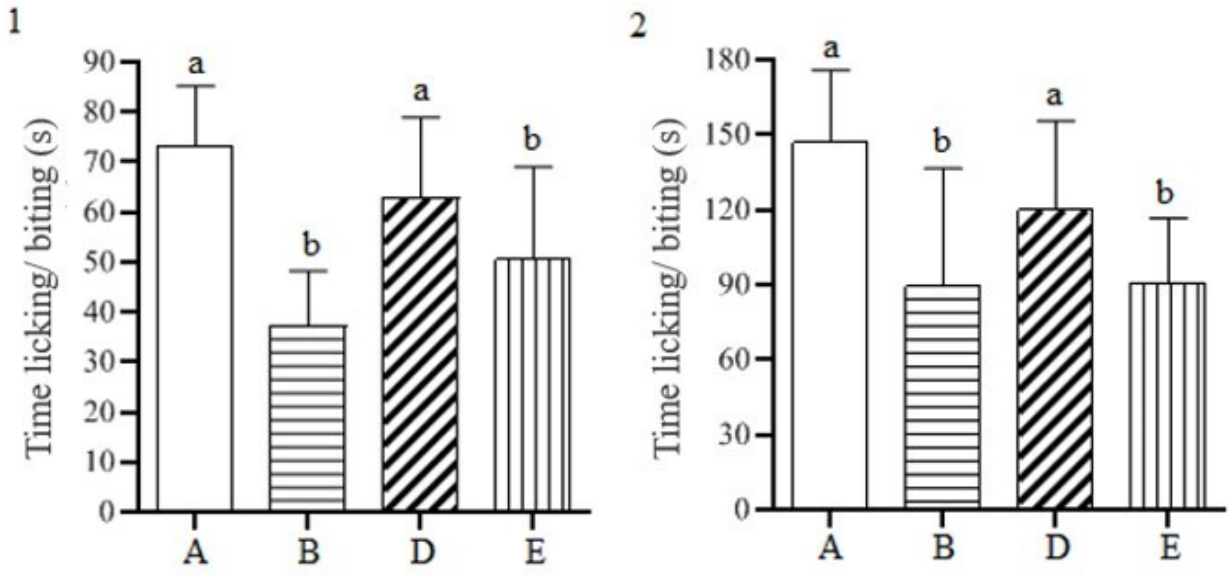

Figure 4. Paw licking and/or biting time in mice treated with morphine, laser therapy, and acupuncture on nociception induced by intraplantar formalin injection, neurogenic and inflammatory phase. Data were expressed as the mean ± SEM from 8 to 10 animals in each group. Different letters indicate a statistical difference between treatments (p<0.05). (1) Phase 1 – neurogenic. (2) Phase 2 – inflammatory. (A) Control. (B) Morphine, 1 mg/kg s.c. (D) Laser therapy at acupoints bl60, li11, st36, and gb34. (E) Acupuncture at acupoints bl60, li11, st36, and gb34.

an increase in the nociceptive threshold and, consequently, a therapeutic response to the hot plate. The results found by Goldman et al. (2010) showed that adenosine, a neuromodulator with anti-nociceptive properties, was released during acupuncture in mice and that its anti-nociceptive actions required adenosine A1 receptor expression.

In addition, to evaluate the effect of acupuncture and laserpuncture on the inflammatory process, the paw edema experiment using carrageenan was carried out in this study. Carrageenan-induced edema is a widely used test to determine the anti-inflammatory activity of various therapeutic substances and procedures. The mediators that take part in this inflammatory process are already widely characterized (Posadas et al. 2004, Cotran et al. 2005, Chiu et al. 2012).

Currently, several drugs are available for clinical use in case of pain/inflammation, such as analgesics and/or anti-inflammatories, such as corticosteroids, opioids, and NSAIDs. Among them, glucocorticoids, such as Dexamethasone, have a wide range of pharmacological actions, with anti-inflammatory and immunosuppressive effects, inhibiting both early and late manifestations of the inflammatory process (Gilroy et al. 2004). This supports the results of this study, in which mice treated with Dexamethasone showed a decrease in carrageenan-induced inflammation.

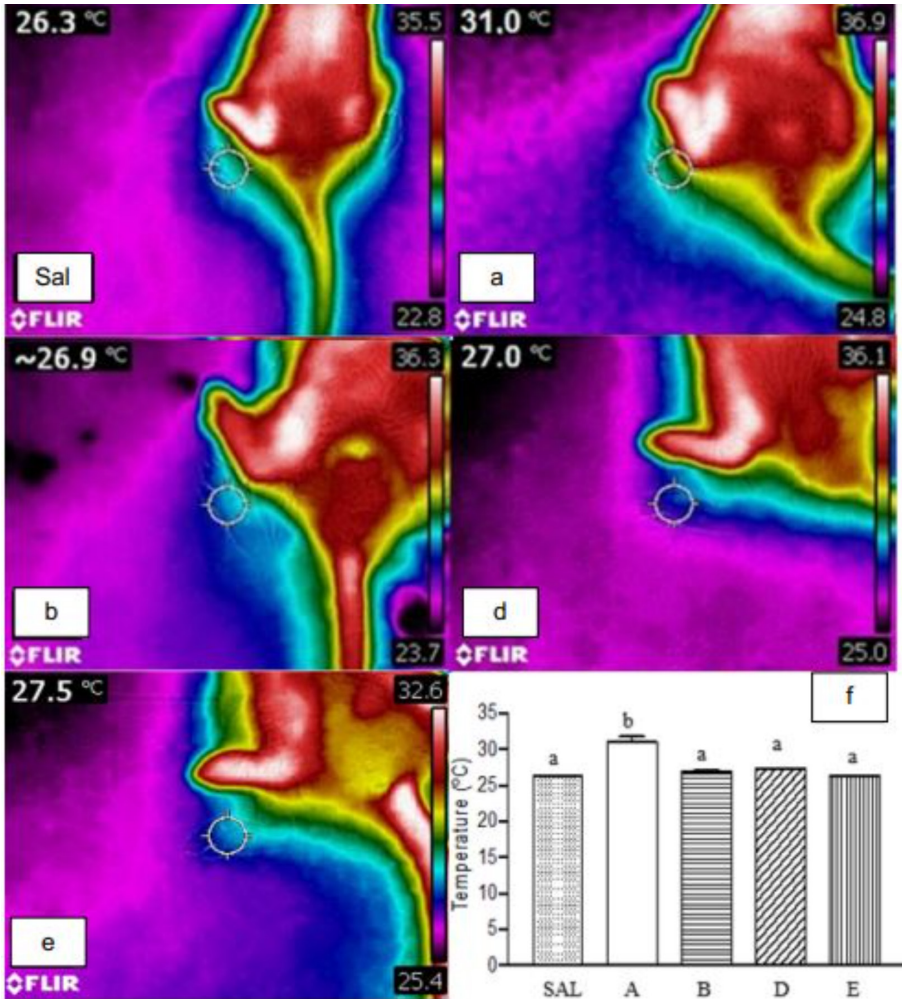

Figure 5. Mean temperatures and representative thermal image of the left hind paw of *Mus musculus* mice treated with morphine, laser therapy, acupuncture, formalin, and control negative (saline solution). (Sal) Negative control (saline solution). (a) Formalin. (b) Morphine, 1 mg/kg. (d) Laser therapy at acupoints bl60, li11, st36, and gb34. (e) Acupuncture at acupoints bl60, li11, st36, and gb34. (f) Average temperature of experimental groups. Data were expressed as the mean ± SEM from 8 to 10 animals in each group. Different letters indicate a statistical difference between treatments ($p<0.05$). Warm colors (green, orange, red, pink, and white) indicate an increase in temperature; cool colors (blue and purple) indicate a decrease in temperature.

However, despite these classes of substances having excellent anti-inflammatory properties (except for opioids) and being used in clinical therapy, their use produces important side effects (Gilroy et al. 2004). This fact has encouraged us to search for therapeutic alternatives with fewer undesirable effects, such as laser irradiation and acupuncture.

In fact, the results of this work show a decrease in the inflammatory process, induced by carrageenan, in mice treated with laserpuncture and acupuncture. The effects generated by laser irradiation are photo-stimulating due to the increase in cell metabolism, cell chemotaxis, and vascularization. Some studies that used LED light in joints showed a decrease in the rate of mRNA for TNF-α (Xavier et al. 2010, Oshima et al. 2011) IL-1β, IL-6, as well as for COX-2, with consequent inhibition of prostaglandin E2 synthesis (Xavier et al. 2010, Lim et al. 2007), which may indicate a potential of this phototherapy in the treatment of inflammatory processes, similar to what happened in the results of this work.

Regarding the positive effects of acupuncture on inflammation observed in this research, the manipulation of the acupuncture needle causes a deformation of the connective tissue, composed of extracellular matrix and collagen fibers, with the transmission of a mechanical signal within fibroblasts and other cells adhered to the collagen fibers and a cytoskeletal rearrangement cellular response, stimulating various mechanoreceptors and/or nociceptors (Langevin et al. 2001).

The participation of polymodal receptors has been evidenced in the peripheral mechanism of acupuncture. These receptors respond to mechanical, chemical, and thermal stimuli and consist of free nerve endings. They are located in different tissues and might be sensitized and present in trigger points. Many trigger points are known to correspond to acupuncture points (Kawakita & Okada 2006).

In the study carried out by Erthal et al. (2016) in which the laser was used at point ST36 (Zusanli), a reduction in paw edema induced by carrageenan was observed with inhibitions of 13% at 2h, 18% at 3h, and 12% at 4h, compared to the control group. The study by Lima (2008) used acupuncture on the BL60 acupoint (Kunlun) for 10 minutes and was able to inhibit the first phase of the inflammatory process (4h) of the peritoneal cavity induced by the intraperitoneal injection of 0.5 ml of carrageenan solution.

In this context, the results obtained in this study provide physiological, biochemical, and pharmacological evidence to justify the effectiveness of acupuncture and laser irradiation on acupoints ST36, BL60, LI11, and GB34, confirming their analgesic and anti-inflammatory therapeutic potential, as monotherapy or in association with other therapies.

Formalin was also used in this study, a model that induces nociception in two phases, neurogenic and inflammatory. Neurogenic pain, the first phase (0 to 5 minutes), is caused after direct activation of peripheral nociceptors, such as C and Aδ fibers, and produces a characteristic behavior indicative of pain. Inflammatory pain, the second phase (15 to 30 min), occurs after the release of some mediators, causing sensory facilitation and central sensitization of the spinal cord (Tjølsen et al. 1992).

In this work, morphine 1 mg/kg was able to decrease both phases of nociception in mice. Likewise, acupuncture at acupoints BL60, LI11, ST36, and GB34 decreased both phases of nociception. In this sense, these treatments inhibited processes related to the activation of molecules and substances necessary to trigger nociception in both phases.

Several models try to explain the possible mechanisms of action for analgesia through acupuncture, with a neurohumoral model being known, in which the acupuncture needle stimulates the Aδ afferents that end in the dorsal horn of the spinal cord, transferring the impulse to a second neuron within the same spinal segment, activating three levels of the nervous system. In ascending order, these levels are the spinal cord within the same segment, supraspinal region-periaqueductal gray matter, raphe nucleus magnus, and pituitary-hypothalamus complex. When each of these levels is stimulated, specific endorphins and monoamines, serotonin and adrenaline become involved in a chemical cascade that inhibits pain (Yang et al. 2008, Yamamura 2001).

Confirming the results presented in this research with the literature, Lima (2008) showed that stimulation with needles of the Kunlun acupoint (BL60) for 10 minutes produced inhibition of the tactile allodynia induced by the administration of CFA (Complete Freund's Adjuvant) in mice. This confirmed the study by Li et al. (2005) who, through the electroacupuncture technique applied to the Kunlun (BL60) and Zusanli (ST36) acupoints in mice submitted to intraplantar injection of CFA, observed an antinociceptive and anti-edema effect.

Although the statistical analysis did not show differences in the group treated with laserpuncture on formalin-induced nociception, a decrease in both phases was observed in the irradiated mice when compared to the control. This statement can be confirmed when observing the decrease in temperature observed by thermographic analysis of the paw of mice treated with laser (figure 4 1-2).

Infrared thermography, also known as thermography or cutaneous thermography, is a diagnostic method that captures and records the emission of heat from the surface of the body, which changes in face of different physiological or pathological states (Brioschi 2012). It has been used as a tool to document and investigate sharp pain and inflammatory processes (Brioschi 2012, Bandeira et al. 2012).

The results of the present work point to a reduction in skin temperature on the plantar surface of the hind paw of mice treated with laser, acupuncture, and morphine in an acute inflammatory model with formalin, suggesting a potential anti-inflammatory effect. These results corroborate the study by Erthal et al. (2016).

Probably, the temperature reduction caused by laser treatment may have occurred due to its anti-inflammatory effect and/or increased peripheral blood flow, promoting a cooling in the superficial tissue (Balestra et al. 2011). These results are unprecedented, as no other studies using the same methodologies were found in literature.

Finally, it is important to note that, considering the number of studies available on acupuncture and laserpuncture and the attempts to clarify their mechanism of action, there is no doubt that this therapeutic resource can be useful, alone or as an adjuvant, for the treatment of the most varied pathologies. In this way, the present study demonstrated that acupuncture and laserpuncture are potential alternatives that can optimize therapeutic outcomes. As previously described, the acupoints used in this study were chosen, according to traditional Chinese medicine, based on their analgesic and anti-inflammatory effects in humans (Lee et al. 2022). However, until now, few studies had demonstrated the importance of these points in animal models. In fact, we know that such models have fundamental importance for understanding the biological mechanisms involved in their effect (Moré et al. 2021). Therefore, this work provides evidence of the antinociceptive and anti-inflammatory

potential of acupuncture and laserpuncture, as well as paving the way for understanding the possible mechanisms of action involved in the points used.

CONCLUSIONS

1. Acupuncture and laserpuncture at acupoints ST36, BL60, LI11, and GB34 and acupuncture alone at acupoint BL60 showed antinociceptive effects equivalent to morphine 1 mg/kg on nociception caused by hot plate hyperalgesia.

2. Acupuncture and laserpuncture at acupoints ST36, BL60, LI11, and GB34 showed an anti-inflammatory effect equivalent to dexamethasone 0.5 mg/kg in the phase of paw edema induced by carrageenan.

3. Acupuncture at acupoints ST36, BL60, LI11, and GB34 showed an antinociceptive effect equivalent to morphine 1 mg/kg on formalin-induced nociception, neurogenic and inflammatory phase.

4. Acupuncture and laserpuncture at acupoints ST36, BL60, LI11, and GB34 showed an anti-inflammatory effect equivalent to morphine 1 mg/kg, decreasing the temperature of the plantar surface of the paw of mice, observed by thermographic analysis.

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