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Research Article

Transcutaneous Electrical Acupoints Stimulation Attenuates Inflammatory Pain in Rats

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Received: October 17, 2019; **Published:** November 28, 2019

Abstract

Background: Electroacupuncture (EA) has been observed to exerts in inflammatory pain. However, the invasiveness of EA may bring risk of infection. Transcutaneous electrical acupoint stimulation (TEAS)with similar parameters as EA without the risk of infection. Previous studies have shown that TEAS treatment can alleviate postoperative pain. However, its analgesic role in inflammatory pain has not been investigated.

Methods: Complete Freund's adjuvant (CFA)was injected into the male Sprague-Dawley of rats to generate an inflammatory model. EA or TEAS treatment at the *Zusanli* (ST36) and *Sanyinjiao* (SP6) acupoints was given 4 days of CFA injection of mechanical and thermal sensitivity were determined. Microdialysis was performed before and after EA or TEAS. Adenosine concentration of dialysates as measured by high performance liquid chromatography.

Results: TEAS treatment showed similar analgesic effects to EA treatment as assessed of rat's paw withdrawal latency in response to mechanical and thermal stimulation. EA treatment significantly elevated adenosine concentration for at least 30 minutes, while TEAS induced a significant adenosine increase with similar amplitude over 60 minutes.

Conclusion: TEAS may be a more convenient and more effective substitute for EA in inflammatory pain treatment.

Keywords: *Transcutaneous Electrical Acupoint Stimulation; Electroacupuncture; Inflammatory Pain; Analgesia*

Introduction

Inflammatory pain is a major health care problem that dramatically decreases the quality of life of patients. Inflammatory pain results from peripheral tissue injury/inflammation and is accompanied by sensitization to noxious stimuli (hyperalgesia) [1,2]. Both peripheral and central nervous system are involved in the modulating on of pain nociception under local or systemic inflammatory conditions. Tis-

sue injury leads to the release of inflammatory mediators that sensitize and activate peripheral primary nociceptive neurons, resulting in the increased sensitivity of neurons in the dorsal horns of the spine, eventually central sensitization Peripheral receptor modulation and central sensitization contributes importantly to the development and maintenance of inflammatory pain [3]. Inflammatory pain is generally treated with opioids, nonsteroidal anti-inflammatory drugs (NASIDs), paracetamol and steroids. However, these drugs are limited by their lower efficacy and/or accompanying side effects; thus there is a significant unmet clinical need for other treatments for inflammatory pain [4].

Acupuncture has been used for over 3000 years in Asia to treat pain and the analgesic efficacy of acupuncture is recognized worldwide. Electroacupuncture (EA)is a form of acupuncture which involves passing a small electric current between pairs of acupuncture needles [5]. EA is considered an adjunct to augment the use of regular acupuncture and several clinical trials have demonstrated that effects of EA analgesic a involves many different factors such as peripheral and central nervous system humoral regulation. EA blocks pain by activating a variety of bioactive chemicals through peripheral, spinal and supraspinal mechanisms [5,6]. EA intervention has demonstrated effectiveness in alleviating neuropathic pain in Chronic Constriction Injury (CCI) rats, which may be closely related to its effects in lowering the functional activity of NR 1 protein and mRNA in the spinal cord [7]. Goldman's group have demonstrated that acupuncture promotes the peripheral release of adenosine, which exerts an antinociceptive effect through A1 receptors (A1Rs) [8]. Therefore, EA may be a new complementary and alternative therapy for inflammatory pain.

TEAS is a form of non-invasive electrical stimulation that produces a perceptible sensation through electrodes placed on acupoints. Compared with conventional acupuncture, the skin is not punctured, so it has no risk of infection and TEAS can potentially be applied by medical personnel with minimal training [5,9]. It has been shown that TEAS reduces the consumption of anesthetics during surgery, reduces the incidence of side effects often arising with general anesthesia and improves postoperative recovery [10-12]. Furthermore, TEAS can reduce the intensity of postoperative pain and the need for perioperative analgesia in colonoscopy [13], lumbar spine surgery [14], ambulatory breast surgery [10], supratentorial craniotomy [15] and video-assisted thoracic surgical lobectomy [12]. There has, however, been no study published which assessed the effects of TEAS on inflammatory pain and the underlying mechanisms.

This study was designed to compare the analgesic effects of TEAS and EA in treating inflammatory pain induced using Freund, Peripheral local changes in levels of adenosine, an important analgesic molecule, after EA or TEAS treatment were examined.

Materials and Methods

Animals

Sixty male specific pathogen-free Sprague-Dawley rats weighing 200 - 250g, were provided by the Experimental Animal Center of Sun Yat-sen University (Guangzhou, China). They were housed at the center throughout the experiments at 23 ± 1°C and 55 ± 5% relative humidity with free access to food and water. A 12h:12h light:dark cycle was maintained (lights on at 07:00). The animals were labelled from 1 to 60 and randomized by a computer program into four groups (n = 15 in each): a vehicle+sham EA group, a CFA+sham EA group, a CFA+EA group, and a CFA+TEAS group. All of the animals were treated in accordance with the Guidelines for Animal Experimentation of Sun Yat-sen University and the study protocol was approved by the university's Ethics Committee. All efforts were made to minimize animal suffering and the number of animals used.

CFA pain model

To induce inflammatory pain, rats were placed under isoflurane anesthesia (5%) and 100 μL of Complete Freund's Adjuvant (CFA) was injected into the plantar side of the right hindpaw. In the sham group the rats received the same volume of normal saline.

Electroacupuncture treatment

The rat analogues of the *Zusanli* (ST36) and *Sanyinjiao* (SP6) acupoints were located according to the Animal Experimental Acupuncture Points Diagram published by the Association of Experimental Acupuncture Research of China's National Association of Acupuncture.

ST36 is in the anterior tibial muscle approximately 1/6 up the length of the lower leg below the knee [16]. SP6 is located 0.2 cm superior to the tip of the medial malleolus, the rear edge of the medial tibia [17]. EA was applied using Huatuo stainless steel needles (0.25 mm × 25 mm, Suzhou Medical Instruments, Suzhou, China) inserted 7 - 10 mm into the two muscles. The electrical stimulation was generated by a HANS-200E electroacupuncture apparatus (Nanjing Jisheng Medical Technology, Nanjing, China). EA was administered 4 days after the CFA injection. The stimulation intensity was alternately 2 Hz and 100 Hz and the current intensity was 2 mA, once a day, each EA session lasted 30 minutes, treatment of four times.

Transcutaneous electrical acupoint stimulation

The transcutaneous stimulation was also administered by an experienced acupuncturist to ensure correct positioning. The electrical stimulation was generated by a HANS-200A electroacupuncture apparatus (Nanjing Jisheng Medical Technology, Nanjing, China). The electrodes were placed over the same two acupoints (ST36 and SP6), the treatment regimen was same as for EA treatment.

Behavior

The Baseline pain threshold were measured before injection of saline or CFA (D0), and the second test (D4) after injection of 4 days, Sham electroacupuncture/electroacupuncture treatment for 30 minutes after second test, and Quiet in the measuring instrument pain after 5~10 minutes was measured for the third test. Testing 10 randomly-selected rats from each group. The test were performed in a room at approximately 25°C when the animals were calm. Thermal pain (Hargraves'test)was measured with a Plantar Test 7370 analgesiometer (Ugo Basile, USA) according to Hargreaves., *et al* [18]. Using heat radiometer the light spot vertically irradiated the skin between the second and third toes of the right hind foot of the rat, from the spot irradiation to the right limb raise the foot escape response time is paw withdraw latency (PWL) and the PWL mean value was recorded after 3 exposures. Mechanical sensitivity was measured with MD-2000 von Frey filaments (BASi, USA) according to DIXON [19], VonFrey fibers with different folding forces were used to stimulate the external side (sural nerve of the paw metatarsal) and the inner surface (saphenous nerve), at different pressures from small to large with vertical stimulation, progressively pressurized to bend the filaments into an s-shaped kept for 6 - 8 seconds to observe whether the rats shrank feet during the stimulation, if a quick foot lift to avoid stimulation is a positive reaction will be able to induce 5~6 times above the fibrillar fold of retraction reflex, the force as the mechanical pain threshold.

Microdialysis

The remaining 5 rats from each group were used for microdialysis experiments on day 5. An MD-2000 microdialysis probe (BASi, USA) was inserted 3 - 4 mm into the center of the right musculi tibial anterior guided by a syringe needle. It was thus near the ST36 acupoint. The probe was secured in place with an MR-5314 binder (BASi, USA). Ringer's solution was infused at a rate of 1μl/min. The inlet and outlet dialysis capillaries were connected through polyethylene tubing. The inlet tube was connected to an MD-1001 microsyringe (BASi, USA) driven by an MD-1000 microdialysis pump (BASi, USA). The outlet led into a collection tube in an ice bath. Dialysates were collected at 30 minutes before treatment and 0, 30, 60 and 90 minutes afterward.

Chromatographic analysis

High-performance liquid chromatography (HPLC)was performed using a 4.6 × 150 mm column (Yi Lite, Dalian, China) filled with 5 μm Hypersil Octadecylsilyl (ODS). Methanol was used as mobile phase A.A 0.214M KH_2PO_4 solution buffered with 2.3 mM tetrabutylammonium hydrogen sulfate to pH 5.88 was used as mobile phase B. The feed rate was 1 mL/min at 30°C. The sample volumes were 20 μL. Adenosine the dialysates was detected using a UV detector measuring the absorbance at 266 nm.

Statistical analysis

The will be reported as mean ± SEM (standard error of the mean). The significance of the total difference between the groups was computed using two-way analysis of variance (ANOVA), with multiple comparisons by Tukey's test. All of the statistical analyses were performed using IBM's SPSS statistics software (version 22.0). A $p \leq 0.05$ was considered the threshold of statistical significance.

Results

TEAS significantly reduced pain

Neither mechanical or temperature sensitivity was different among the four groups on average before the experiments. Compared with vehicle injection, CFA injection significantly reduced the rats average paw withdrawal latency in response to mechanical or thermal stimulation. As figure 1 shows, the EA treatment tested also significantly increased the average paw withdrawal latency in response to mechanical or thermal stimulation. That was to be expected, but more interesting, TEAS treatment showed analgesic effects similar to those of EA treatment.

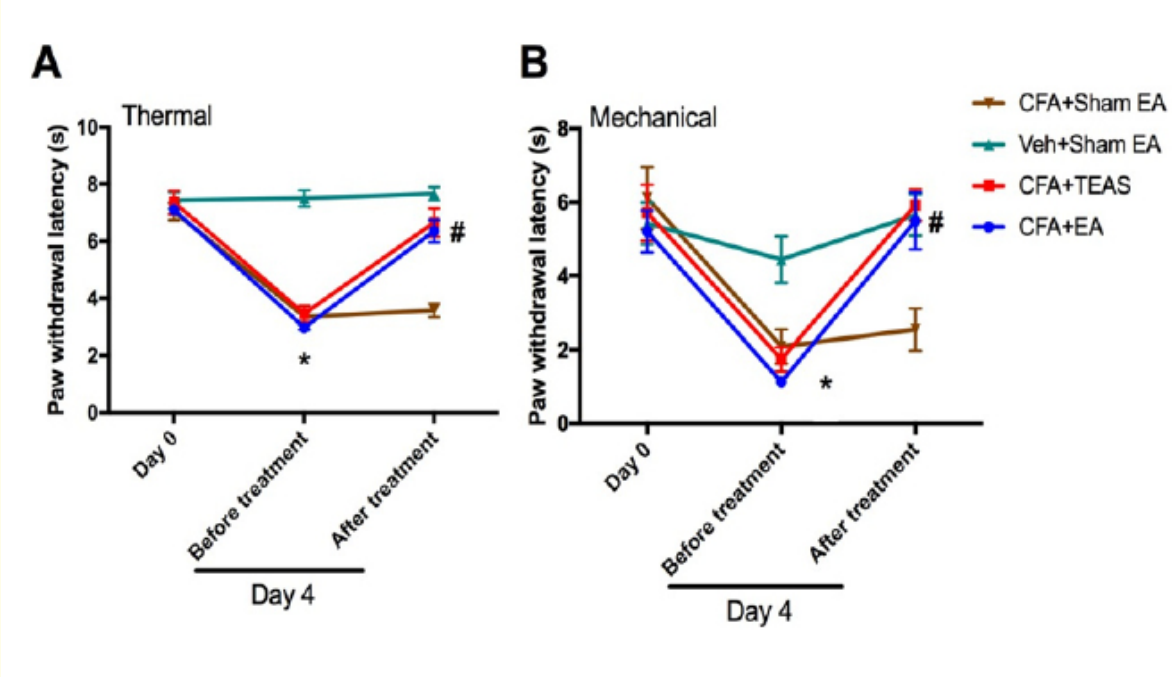

Figure 1: *Effects of EA and TEAS on CFA-induced mechanical allodynia (A)and thermal hyperalgesia (B). *Indicates a difference from the veh+sham EA group significant at the p ≤ 0.05 level of confidence. #Indicates a difference from the CFA+sham EA group significant at the p ≤ 0.05 level of confidence. Veh: Vehicle.*

TEAS increased local adenosine levels

It has been reported that adenosine receptor activation is a critical element in the analgesia of inflammatory pain [8], but local adenosine levels have seldom been investigated in inflammatory pain studies. Figure 2 show that the peripheral adenosine concentrations in the CFA-injected rats was significantly lower, on average, than those observed in the saline-injected rats. EA treatment significantly elevated local adenosine concentration near the acupoint, and that elevation lasted at least 30 minutes. TEAS treatment increased adenosine similarly and that elevation lasted more than 60 minutes.

Discussion

This study compared EA and its substitute TEAS for treating CFA-induced inflammatory pain. As in previous studies [6,20], EA treatment significantly reduced nociceptive responses of rats to mechanical or thermal stimulation and increased the local concentration of

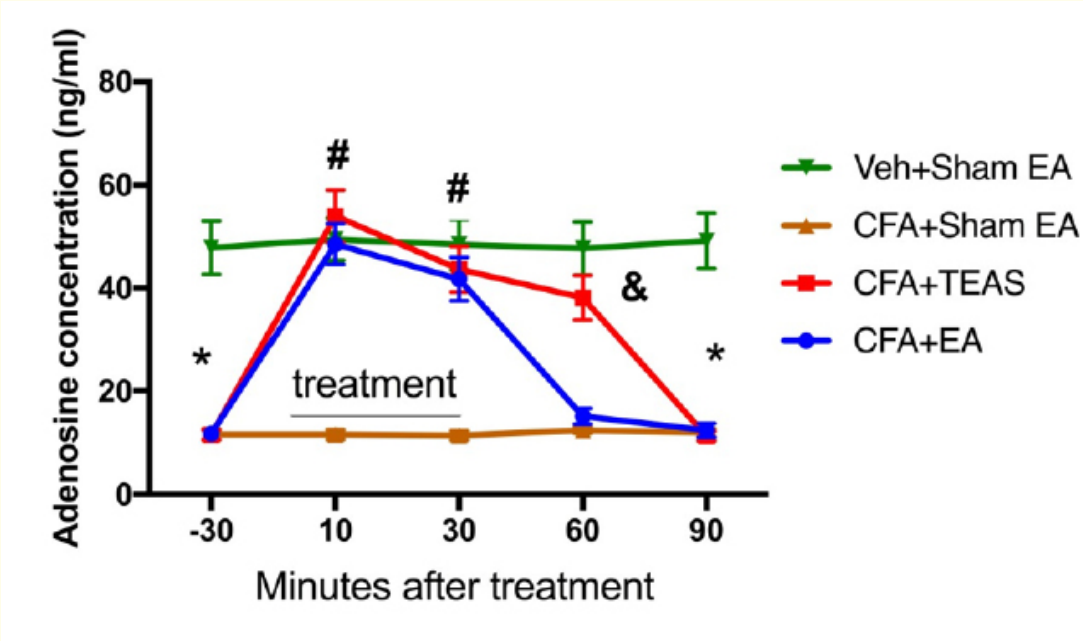

Figure 2: *Effects of EA and TEAS on adenosine concentration. *Indicates a difference from the veh+sham EA group significant at the p ≤ 0.05 level of confidence. #Indicates a difference from the CFA+sham EA group significant at the p ≤ 0.05 level of confidence and compared with the CFA+sham EA group for the CFA+TEAS group. Veh: Vehicle.*

adenosine, an important analgesic molecule. TEAS treatment had similar analgesic effects, but it induced longer-lasting adenosine elevation than EA. These findings suggest that TEAS may be a better substitute for EA in inflammatory pain treatment.

EA treatment does, after all, have several limitations. The main risk is of infection at the acupoints. In addition, some patients fear the invasive needle operation. Compared with acupuncture or EA, TEAS is a non-invasive. There is no risk of infection or reason to fear. TEAS is thus more 'user friendly'. And this study has shown that it has analgesic effects similar to those of EA treatment. Moreover, there have been clinical studies showing that TEAS is an effective and safe adjunctive for management of postoperative pain [21]. So the data collectively indicate the promising application of TEAS in clinical pain treatment.

The mechanisms underlying the analgesic effects of EA and TEAS remain unclear. There are many theories about the mechanisms underlying the analgesiceffect of acupuncture treatment, including the gate control and the endogenous opiates theories [22]. However, peripheral deregulation of opioids alone cannot completely explain the analgesic effects of acupuncture. Adenosine receptors are widely distributed throughout the pain signaling network, and adenosine systems constitute an endogenous regulator which can potentially be recruited by pharmacological agents to produce anti-nociception [23]. There are four types of adenosine receptors: A1R, A2AR, A2BR and A3R. A1R mediates antinociceptive effects through both the central and peripheral nervous systems, whereas A3R exerts its analgesic effects via glial cells in several neuropathic pain models. In contrast, both A2AR and A2BR exhibit two-sided effects on pain modulation-peripheral nociception and spinal anti-nociception. Treatment with an agonist of adenosine receptor A1Rreduces CFA-induced mechanical and thermal hyperalgesia [24] and adenosine now has been identified as a significant contributor to peripheral mechanisms involved

in acupuncture's analgesia [23]. Blocking A1R with antagonists or A1R gene knockout significantly reduces the analgesic effects of acupuncture treatment [8,24,25].

Consistent with the findings of previous studies [25], this study has shown that both EA and TEAS treatment increase local adenosine levels, which would activate A1R signaling to mediate analgesic effects. That TEAS treatment was observed to induce longer-lasting increases in adenosine levels than EA suggests its promise for application in pain treatment.

This study also found that CFA-induced inflammation caused a greater decrease in peripheral adenosine than vehicle. That contradicts the findings of previous studies to the effect that peripheral adenosine release can be triggered by both formalin-induced inflammation and nerve injury [26]. The discrepancy might be due to differences in the composition of the inflammatory substances tested CFA is a more effective stimulant of immune stimulation functions, whereas formalin mainly causes tissue injury. Further studies are needed to explore the different mechanisms of adenosine release comparing different inflammation-inducing substances.

It should of course be acknowledged that any effects of TEAS treatment on the levels of other analgesic substances such as opioids were not examined. Such substances might mediate the effects of TEAS. Neither where molecular mechanisms by which adenosine is released investigated in these animal models. The precise adenosine receptors through which TEAS exerts its analgesic effects certainly calls for further study. Previous studies have demonstrated that A1R may be the main mediator of peripheral antinociceptive effects in inflammatory pain [23], but further studies investigating in detail the mechanisms by which TEAS inhibits inflammatory pain are certainly called for.

Conclusion

In summary, this study has shown that TEAS treatment shows antinociceptive effects similar to those of EA in treating CFA-induced inflammatory pain. And it has shown that TEAS induces longer-lasting adenosine increase (an presumably pain relief) than EA. These findings suggest that TEAS may be a more convenient and more effective substitute for EA in pain treatment.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

This study was supported by CAS Key Laboratory of Human-Machine Intelligence-Synergy Systems, Shenzhen Institutes of Advanced Technology (No. 2014DP173025),National Natural Science Foundation of China (No. 81874499),Science Research Project of the Shenzhen Health and Family Planning System(No.SZXJ2018026).

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