Analgesic Effect of Electroacupuncture on Complete Freund’s Adjuvant-Induced Inflammatory Pain in Mice: A Model of Antipain Treatment by Acupuncture in Mice

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Abstract: Electroacupuncture (EA) was applied bilaterally to the acupoints of Zu-san-li (ST-36) and Kun-lun (BL-60) in the hindlimbs of mice. The therapeutic effect of EA on inflammatory pain induced by an ipsilateral injection of complete Freund’s adjuvant (CFA) into the right paw of the mouse was investigated in this study. The time of paw-withdrawal latency (PWL) was used as an indicator for judging the intensity of the pain induced by the CFA injection. The EA effects were divided into immediate (PWL tests within 2 h after EA treatment) and cumulative (PWL tests during and after repetitive EA treatments for 3 weeks) effects. As immediate effects, PWL was significantly shortened in the CFA-injected paw, but was again prolonged 20 min after an EA treatment and lasted until 30 min after. As cumulative effects, PWL was significantly shortened in the CFA-injected paw, but recovered from the 2nd to the 8th day during repetitive EA treatments. No such effects could be observed after sham EA treatment, which resulted in behavior similar to that in untreated animals. These results demonstrate that the CFA-induced inflammatory pain in mice is an ideal model system for the investigation of EA effects and may serve as a valuable reference for the clinical treatment of inflammatory pain in human beings. Furthermore, the mouse pain model opens the possibility to apply the investigation also to transgenic mice. [The Japanese Journal of Physiology 55: 339–344, 2005]

Key words: electroacupuncture, acupoint, inflammatory pain, complete Freund’s adjuvant, paw-withdrawal latency.

The subcutaneous intraplantar injection of complete Freund’s adjuvant (CFA) into the local area of an animal can cause severe inflammatory pain around the injected area [1–3]. This CFA-induced inflammatory pain has been widely used as a kind of pain model in the field of pain research since it was reported [4–6].

Besides clinical treatment by antiinflammatory and antipain medicine, acupuncture can serve as a supplementary or alternative treatment. It has been shown to be effective in treating patients clinically who suffer from inflammatory pain [7–9]. Such an effect of acupuncture on relief from inflammatory pain was recently reconfirmed by an American NIH-supported clinical investigation [10]. This report has pointed out the necessity of applying various medical and biological research techniques in the future to further investigate the action of the acupuncture and the underlying mechanisms. For this it is essential to develop an effective acupuncture-pain-treatment animal model. This was the purpose of our study.

Evidence has accumulated indicating that stimulating different acupoints in the body at different spinal segmental levels could produce various analgesic effects according to different areas where pain occurred [11, 12]. Not only the ancient medical practices in China, but also modern medical treatments
revealed that certain acupoints, such as Zu-san-li (ST-36) and Kun-lun (BL-60), in the legs or hind limbs were effective in treating diseases in the foot, including pain [13, 14]. Such evidence encouraged us to select those acupoints as candidates for our investigation of the therapeutic effects of acupuncture on CFA-induced inflammatory pain in the mouse pain model. 

MATERIALS AND METHODS

Animals and care. Adult male Kun-ming mice weighing 18–22 g were purchased from the Experimental Animal Center, Shanghai Medical College, Fudan University. The mice were housed in plastic cages containing corn-chip bedding with free access to food and water for 3 days after their purchase; thereafter they were used for experiments. During the entire study, all the mice were kept in an environment with a cycle of 12 h light and 12 h dark. All mice in this study were used strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals in order to minimize the number of animals used and their suffering.

Inflammatory model and experimental groups. Inflammatory pain was induced by an injection of 20 µl complete Freund’s adjuvant (CFA, Mycobacterium tuberculosis; Sigma, St. Louis, MO, USA) into the right paw of the mouse. Several tests showed that an injection of this volume of CFA could produce inflammation and tissue swelling in a local area of the right paw. The mice were separated into 4 groups: (1) the saline group (sln) with saline injection (same volume as used for the CFA injection) and receiving no electroacupuncture (EA) treatment; (2) the model group (mdl) with CFA injection and also without EA treatment; (3) the EA group (mdl EA) with CFA injection but treated with EA; (4) the sham EA group (shm EA) with CFA injection and treated by sham EA (just inserting needles into the acupoints without electric stimulation).

EA treatment. The EA treatment was applied by two pairs of stainless steel needles (0.25 mm in diameter) inserted bilaterally at a depth of 3 mm into Zu-san-li (ST-36, 3 mm lateral to the anterior tubercle of the tibia and 4 mm below the knee joint) and 2 mm into Kun-lun (BL-60, at the ankle joint level and between the external malleolus and tendon calcaneus) in the hindlimbs. Each pair of needles (one in ST-36 and the other in BL-60) was connected to the output terminals of an EA apparatus (Model G-6805-1A, Shanghai Medical Electronic Apparatus Company, China). Trains of electric pulses with dense-sparse frequencies (100 Hz for 1.05 s and 4 Hz for 2.85 s, alternately), approximately 1 mA in intensity, were selected as stimulation parameters for EA treatment, and the stimulation was delivered for 30 min each time. For treatment with sham EA, acupuncture needles were inserted into the ST-36 and BL-60 acupoints without any electric stimulation for 30 min.

Observation of EA effects. The EA effects were divided into immediate and cumulative. The immediate effect meant that data were obtained by performing paw-withdrawal latency (PWL) tests (see below) 5 times within 2 h as soon as the EA treatment was finished. The cumulative effect meant that data were obtained by the tests performed as follows: (1) EA treatments were repetitively applied every other day and lasted for 14 days; (2) PWL tests were performed 24 h after each EA treatment (i.e., on the day without EA treatment), and they were continued until the 21st day, though the EA treatment was terminated after 14 days.

Behavioral test. The PWL was used to assess the inflammatory pain according to the method of Harreaves et al. [15]. The CFA-injected area of the right paw was placed on a constant-intensity radiant heat source (Tail Flick Analgesia Meter, Model 33, IITC Inc., Life Science Instruments, USA), and the time of paw-withdrawal latency was measured by observing the animal’s paw-withdrawal response after applying initial heat to the left or right paws. The intensity of the heat source was set to cause paw-withdrawal response after about 10 s in normal mice (animals similar to those tested in this study, but separately raised for intensity calibration). To avoid burning and excessive suffering of animals, the time for terminating heat administration was set at 20 s even if there was no paw-withdrawal response, and an interval time for repeated tests on the same animal was set at about 10 min.

Assessment of inflammation. Besides the PWL tests, the circumference of the paw around the saline- or CFA-injected point was measured to assess the severity of inflammation. The measurement was done by slightly winding a thread around the paw and reading the thread length. The measurements of the circumference were performed at the same times as the PWL tests for cumulative effects.

Statistical analysis. All data were presented as mean ± SEM. A statistical analysis was performed by repeated ANOVA followed by Fisher’s PLSD procedure for significance (P < 0.05), using StatView (Version 5.0, for Windows) statistical software.
RESULTS

Immediate effects of EA on PWL responses

The immediate and cumulative effects of acupuncture treatment have been reported [16]. To follow up such effects, we also divided the experimental observations after acupuncture treatment into immediate and cumulative. In the immediate observations (Fig. 1), we found that PWLs were significantly shortened on the right side (injected side) after injection (injct) of CFA in the mdl, mdl EA, and shm EA groups, but not in the sln group. The shortened PWL remained during the entire 80 min observation in the mdl and shm EA groups. In the EA-treated mdl EA group, the shortened PWL did recover temporarily in the time period from 20 to 30 min after the EA treatment, but returned thereafter to the shortened PWL time seen for the mdl and shm EA groups. In the sln group, a significantly shortened PWL was observed only 20 min after the saline injection, then returned to its control level (Fig. 1B). There were no apparent changes in PWL on the left side (intact side) in all 4 groups tested (Fig. 1A).

Cumulative effects of EA on PWL responses

Similar to the immediate observation, there was a significant decrease in PWL in the mdl, mdl EA, and shm EA groups after injection (injct) of CFA on the right side (injected side) in the cumulative observation (Fig. 2). The decreased PWL continued during the 14-day lasting observation in the mdl and shm EA groups. After repetitive EA treatments (2 times) the decreased PWL of the mdl EA group recovered at day 8 to the value seen in the sln group (Fig. 2B). There were no apparent changes in PWL on the left side (intact side) in all 4 groups tested (Fig. 2A).

Effect of EA on paw circumferences

Figure 3 shows the changes of paw thickness for the 4 groups of tested mice. Except for the sln group, the circumference of the right paws was significantly increased in all other groups (mdl, mdl EA, and shm EA) and lasted during the 21-day observation period (Fig. 3B). In none of the tested groups could we observe significant changes in the circumference of the left paws (Fig. 3A).
Relation of PWL and paw circumferences

For a further interpretation of the above results on the cumulative observations, we compared PWL times and paw circumferences of the right CFA-injected side for the mdl, mdl EA, and shm EA groups (Fig. 4). In mdl and shm EA mice, the inflammatory swelling (as measured by the paw circumference) continued to increase, which is paralleled by a gradual recovery of the longer PWL time. In contrast, in the mdl EA mice the inflammation is kept on its initial elevated value, and full recovery of the PWL time is gained after a few days.

DISCUSSION

CFA is a potent agent that can produce inflammation of the tissue, and it has been clinically used as a model of peripheral inflammatory pain in animals such as rats and mice representing a kind of peripheral inflammatory disease. An injection of CFA into the right paw of the mouse produced severe inflammation of the local area, such as reddish tissue (data not shown), and swelling, as illustrated by the paw-circumference increase (Fig. 3B). Parallel to these effects, there was severe algesia indicated by, for example, lifting and licking the paw to lessen the pain (data not shown) and a decrease in pain threshold (PWL shortened) in the injected paw (Figs. 1B and 2B).

Electroacupuncture (EA) has been practiced clinically in treating patients who suffer from pain not only in China, but also worldwide. The effectiveness of EA on inflammatory pain was confirmed in the present study in the mouse, which may therefore serve as a pain model for acupuncture research. The data demonstrate that EA can be applied for therapeutic relief from algesia by increasing the pain threshold at the inflammatory area of the paw. Different effects were observed after immediate and cumulative treatments with EA. Immediately after treatment with EA, a short transient period of analgesic effect lasting from 20 to 30 min was observed, consistent with previous reports on humans [17, 18]; at the end of the entire observation period of 80 min, no further EA effect could be detected. The result suggests that the immediate effects of EA on the CFA-induced inflammatory pain are limited. On the other hand, cumulative treatment with EA resulted in a long-lasting analgesic effect during and after repetitive treatments with EA. The effect appeared one day after the mice had received the first treatment with EA; it continued and gradually increased to maximum during the 3 succeeding EA treatments over the next 6 days. The result suggests that repetitive EA treatments could produce more-intensive prolonged effects in relief from algesia induced by the CFA inflammation.

Although our results provide no evidence for an explanation of the differences in the analgesic effects of single and repetitive EA treatments, recent studies have shown that single acupuncture treatment played an analgesic role mainly by activating the endogenous...
opioid system, and repetitive EA treatments relieved algesia by changing central neurotransmission in the pain control system distinct from the endogenous opioid system [19–21]. Which neurotransmission system is involved needs to be further studied, and we plan to employ our mouse model system in this investigation. The recovery form CFA-induced inflammatory pain was reached after 17–21 days, and the cumulative effect of EA had reached the PWL of the sln group after 8 days (Fig. 2B). It would be helpful to design a CFA inflammatory pain model with a recovery period longer than 21 days for better observing cumulative EA effects.

In conclusion, the results obtained from this study provide experimental evidence that the mouse model can be used as a model system for the investigation of the therapeutic effects of EA on inflammatory pain, and this system can be a valuable reference for clinical treatment against inflammatory pain by acupuncture. Since mice are ideal for producing transgenic animals, it can also be used for the investigation of acupuncture effects on particular proteins that are involved in neurotransmission systems.

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