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Electroacupuncture combined with indomethacin enhances antihyperalgesia in inflammatory rats

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Abstract

Our previous study showed that electroacupuncture (EA), an adjuvant to conventional medicine, significantly attenuated hyperalgesia and inflammation. The present study is an evaluation of the potential synergism of EA and a subeffective dosage of indomethacin (INDO) in a rat model. Inflammation and hyperalgesia, manifesting as edema and decreased paw withdrawal latency (PWL) to a noxious stimulus, were induced by injecting complete Freund's adjuvant (CFA) subcutaneously into the plantar surface of one hind paw of the rat. EA treatments were given at acupoint GB30 immediately and 2 h post-CFA. INDO at 2 mg/kg was given (intraperitoneally) 40 min before the second EA. PWL and edema were measured prior to CFA and 2.5 and 5 h post-CFA. Ten and 100 Hz EA significantly inhibited CFA-induced hind paw hyperalgesia. Both low- and high-frequency EA combined with INDO enhanced antihyperalgesia compared to each component alone, and 10 Hz but not 100 Hz EA significantly reduced CFA-induced hind paw edema. A combination of low-frequency EA and INDO did not show synergistic inhibitory effects on edema. The results demonstrate that EA combined with INDO synergistically inhibits hyperalgesia and suggest an improved treatment strategy for inflammatory pain.

Keywords: Inflammation; Pain; Acupuncture; NSAID; Complete Freund's adjuvant

1. Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) are among the most frequently prescribed drugs for treating inflammatory pain. NSAID treatment is commonly associated with gastrointestinal disturbances, particularly gastrointestinal bleeding, perforation, gastric ulcers and impairment of renal function (Scheiman, 2001; Immer et al., 2003). Additionally, it is documented that as many as 42% of patients use complementary and alternative medicine, including acupuncture, to fulfill their needs in lieu of or as an adjunct to conventional medicine (Eisenberg et al., 1998). Our clinical acupuncture trials on osteoarthritis of the knee showed that electroacupuncture (EA) plus antiinflammatory drugs is more effective than drugs alone or than sham EA control plus drugs (Berman et al., 1999). However, few other studies have investigated the effect of EA–drug combinations in well-controlled experimental settings.

Our previous study with an animal model of inflammatory pain showed that EA of 10 Hz/3 mA or 100 Hz/3 mA at acupuncture point Huantiao (GB30) significantly attenuated hyperalgesia (Lao et al., 2001, 2004). Whether EA and conventional pharmaceutical therapy (e.g., NSAIDs) work additively and/or synergistically has not been fully understood. The present study was designed to investigate the effect of combinations of EA and low doses of indomethacin (INDO), a classic NSAID drug, on inflammatory hyperalgesia and edema in a rat model of inflammatory pain.

2. Methods

2.1. Induction of inflammation

Male Sprague–Dawley rats weighing 280–320 g (Harlan) were kept under controlled conditions (22 ± 0.5 °C,

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relative humidity 40–60%, a 7 a.m. to 7 p.m. alternate light–dark cycle, food and water ad libitum). The animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Maryland School of Medicine.

Inflammation and hyperalgesia were induced by injecting complete Freund's adjuvant (CFA; Sigma, St Louis, MO; suspended in a 1:1 oil/saline emulsion, 0.08 ml, 40 μg *Mycobacterium tuberculosis*) subcutaneously into the plantar surface of one hind paw of the rat using a 25-gauge hypodermic needle (Stein et al., 1988). The inflammation, manifesting as redness, edema, and hyperresponsiveness to noxious stimuli, was limited to the injected paw, appeared shortly after the injection, and lasted for about 2 weeks. Hyperalgesia was determined by a decrease in paw withdrawal latency (PWL) to a noxious thermal stimulus.

2.2. Experimental design

Rats were divided into the following seven groups ($n = 7–9$ per group): (1) INDO (Sigma), 2 and 4 mg/kg (0.5 ml ip), (2) vehicle control (0.5 ml ip), (3) INDO (2 mg/kg) plus 10 Hz EA, (4) vehicle plus 10 Hz EA, (5) INDO (2 mg/kg) plus 100 Hz EA, (6) vehicle plus 100 Hz EA, and (7) sham EA (neither electrical stimulation nor manual manipulation). The INDO was dissolved in 0.1% sodium carbonate and administered (intraperitoneally) 40 min before the second EA treatment.

2.3. Acupuncture treatment

EA parameters of 10 or 100 Hz at 3 mA and 0.1 ms pulse width, which showed significant antihyperalgesic effects in our previous studies with the rat inflammation model (Lao et al., 2001, 2004), were used in the present study. The equivalent of human acupoint GB30 (O'Connor and Bensky, 1981) on the rat's hind limbs was treated bilaterally. GB30 was chosen based on traditional Chinese medicine (TCM) meridian theory (O'Connor and Bensky, 1981), its successful use in our previous studies, and its use in studies by others (Xu et al., 1993; Lao et al., 2001, 2004). Our previous study (Lao et al., 2004) demonstrated that EA at acupoint GB30, but not at Waiguan (also known as the fifth acupoint on the Triple Energizer Meridian) or sham points, including the opposite aspect of GB30 and an abdominal point, showed significant antihyperalgesia. In humans, GB30 is located at the junction of the lateral 1/3 and medial 2/3 of the distance between the greater trochanter and the sacral hiatus; underneath are the sciatic nerve, inferior gluteal nerve and gluteal muscles (Cheng, 1987). The comparable landmarks were used to locate GB30 in the rats. The animals were gently handled for 30 min each day for 2–3 days and habituated to the acupuncture treatment before the experiment. After cleaning the skin with alcohol swabs, disposable acupuncture needles (gauge #32, 0.5 in. in length) with electrodes soldered to their handles were swiftly inserted bilaterally, approximately 0.5 in. deep, into GB30 by one investigator while another gently held the animal. The needles and the electrodes were stabilized with adhesive tape (Lao et al., 2004). To prevent the animal from biting the electrical leads, the electrical wires were stabilized on the animal's back, then threaded through a hole on the inverted clear animal cage and connected to the electrical stimulator. The procedure typically lasted less than 20 s and caused little distress to the animal. EA stimulation was delivered by an electrical stimulator (A300 Pulsemaster, World Precision Instruments) via an isolator (A360D Stimulus Isolator, World Precision Instruments) which converts electrical voltage into electrical current. While EA frequency was held constant, intensity was adjusted slowly (over the period of approximately 2 min) to the designated level of

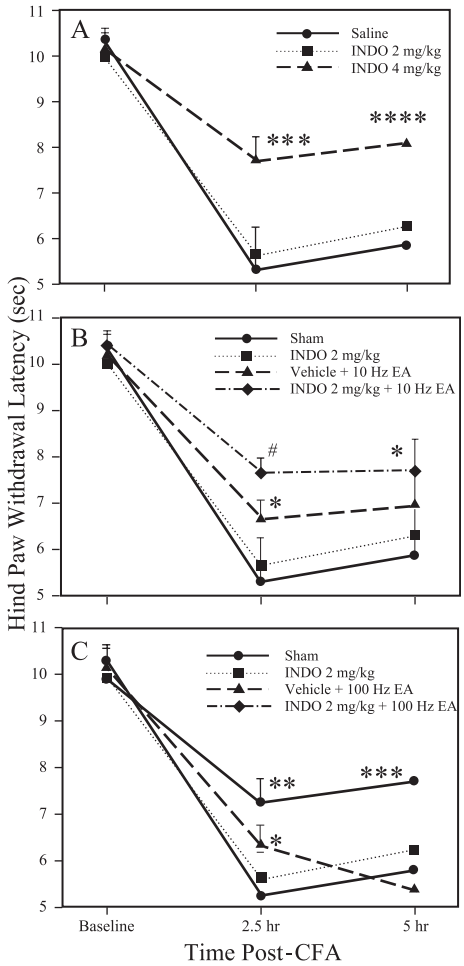

Fig. 1. Effects of EA and INDO on CFA-induced hyperalgesia. (A) INDO at 2–4 mg/kg ip dose-dependently inhibited hyperalgesia. (B) A combination of 10 Hz EA and 2 mg/kg INDO more significantly inhibited hyperalgesia than lower dosages of INDO and than EA alone. (C) The combination 100 Hz EA and 2 mg/kg INDO significantly inhibited hyperalgesia compared to lower dosages of INDO and to 100 Hz EA alone. $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$ and $^{****}P<.0001$ compared to vehicle (in A) or sham treatment (in B and C); $^{\#}P<.05$ compared to vehicle + 10 Hz EA.

3 mA, which is the maximum EA current intensity that a conscious animal could tolerate. Mild muscle twitching was observed. To maximize the antihyperalgesic effect and treat animals prophylactically, EA treatment was given twice, 20 min for each once immediately after administration of the CFA and again 2 h post-CFA. During EA treatment, each rat was placed under an inverted clear plastic chamber (approximately 5 × 8 × 11 in.) but was neither restrained nor given any anesthetic. The animals remained awake and still during treatment and gave no observable signs of distress. For sham control, acupuncture needles were inserted bilaterally into GB30 without electrical stimulation or manual needle manipulation. Sham EA showed little antihyperalgesic effect in our previous study (Lao et al., 2001, 2004) and seems to be appropriate control for nonspecific needling effects.

2.4. Behavioral test

Rats were tested for hind paw thermal hyperalgesia by a method previously developed (Hargreaves et al., 1988; Lao et al., 2001). The rats were placed under a clear plastic chamber on the glass surface of the Paw Thermal Stimulator System (UCSD, San Diego) and allowed to acclimatize for 30 min. A radiant heat stimulus was applied from underneath the glass floor with a high-intensity projector lamp bulb (CXL/CXR, 8 V, 50 W). The heat stimulus was directed onto the plantar surface of each hind paw, and the PWL to the nearest 0.1 s was automatically determined. The intensity of the thermal stimulus was adjusted to derive an average baseline PWL of approximately 10.0 s in naive animals. A 20-s cutoff was used to prevent tissue damage (Hargreaves et al., 1988; Lao et al., 2001). Mean PWL was established by averaging the latency of four tests with a 5-min interval between each test. PWL measurements were made pre-CFA and at two designated intervals post-CFA injection: 2.5 and 5 h. Edema (hind paw thickness) was measured with a caliper at these same time points. The investigator who conducted the measurement was blind to the treatment assignments.

2.5. Statistical analysis

The results are presented as mean ± S.E.M. (Figs. 1 and 3) or percent change ± S.E.M. (Fig. 2). The percent changes in Fig. 2 are presented as (PWLs of experimental groups − PWLs of control group)/(PWLs of control group) × 100%. The actual PWL data were used for two-way analysis of variance (ANOVA) followed by the Dunnett's post hoc test. $P < .05$ was considered significant in all cases. The effect of the combination of EA and drug was compared to the sum of the effect produced by drug or EA alone to determine whether the effect was synergistic or additive. A synergistic effect was defined as the effect of the EA–drug combination being significantly greater than the sum of each individual treatment given alone, while an additive effect was defined as the effect being equal to the sum of each individual treatment given alone (Klaassen, 1996).

3. Results

3.1. Antihyperalgesic effects of EA

Before CFA injection, there were no significant differences in the overall mean baseline PWLs to noxious heat stimuli among all groups of rats, as well as in PWLs

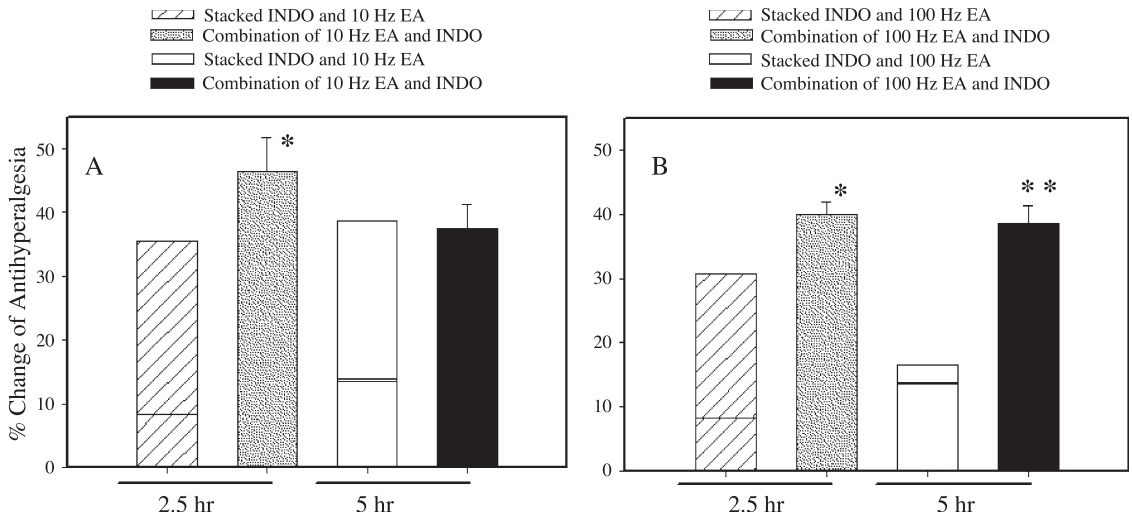

Fig. 2. Additive and synergistic effects of EA and INDO (2 mg/kg) on CFA-induced hyperalgesia. Antihyperalgesia is presented as (PWLs of experimental groups – PWLs of control group)/(PWLs of control group) × 100%. A combination of 10 Hz EA and subeffective INDO enhanced antihyperalgesia 2.5 h post-CFA, while the combination of 100 Hz EA and INDO enhanced antihyperalgesia at 2.5–5 h post-CFA * $P < .05$ and * * $P < .01$ compared to stacked effects at 2.5 and 5 h post-CFA, respectively.

between the left and right hind paws. Following injection of 0.08 ml CFA into the left hind paw, the latency of the injected hind paw was significantly shorter than that of the contralateral hind paw, which remained the same as before CFA. EA at 10 and at 100 Hz significantly increased PWL of the CFA-injected hind paw, indicating an antihyperalgesic effect at 2.5 h post-CFA injection compared to sham treatment (Fig. 1).

3.2. Effects of the combination of EA and INDO on hyperalgesia

INDO induced a dose-dependent antihyperalgesic effect (Fig. 1A): 4 mg/kg significantly inhibited the CFA-induced hyperalgesia while 2 mg/kg had limited effects. A combination of 10 Hz EA and the subeffective dose of 2 mg/kg INDO not only enhanced antihyperalgesia compared to10 Hz EA with vehicle at 2.5 post-CFA ($P<.05$) but also extended significant antihyperalgesia up to 5 h post-CFA injection ($P<.05$, Fig. 1B). The combination of 100 Hz EA and 2 mg/kg INDO also enhanced antihyperalgesia compared to 100 Hz EA with vehicle at 2.5 and 5 h post-CFA injection, while 100 Hz EA with vehicle showed antihyperalgesia only at 2.5 h post-CFA (Fig. 1C).

To discern the additive or synergistic effects of EA combined with INDO, antihyperalgesia was expressed as (PWLs of experimental groups − PWLs of control group)/(PWLs of control group) × 100% and summarized in Fig. 2. Antihyperalgesic effects produced by the combination of 10 Hz and INDO were significantly greater than the sum produced by either 10 Hz or INDO alone at 2.5 h post-CFA, indicating a synergistic effect. At 5 h post-CFA, these combinations showed the same antihyperalgesic effects as those produced by either 10 Hz or INDO alone, indicating an additive effect (Fig. 2A). The combination of 100 Hz and INDO showed significantly greater antihyperalgesia than the sum effects produced by either 100 Hz or INDO alone at 2.5–5 h post-CFA, indicating a synergistic effect (Fig. 2B).

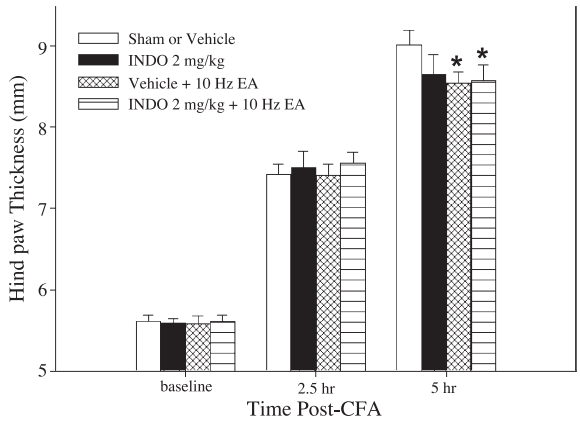

Fig. 3. Effect of EA and INDO (2 mg/kg ip) on CFA-induced hind paw inflammation. Note that the inhibitory effects on edema of EA and INDO alone and in combination are comparative. Sham and vehicle groups showed the same value of hind paw thickness and were presented as one column. * $P<.05$ compared to sham.

3.3. Antiinflammatory effects of EA

As shown in Fig. 3, INDO at 2 mg/kg reduced hind paw edema at 5 h post-CFA, although not with statistical significance compared to vehicle control. EA at 10 Hz, but not 100 Hz, significantly inhibited edema at that time point compared to sham EA control ($P<.05$). No further reduction of paw edema was observed from the combination of 10 Hz EA and INDO (2 mg/kg).

4. Discussion

The results of this study show that the combination of a subeffective dose of INDO and EA treatment produces a level of antihyperalgesia that is greater than either agent alone, as well as the sum effects of the individual treatments. Moreover, acupuncture produces few side effects while NSAIDs often have unpleasant ones: the combination of a subeffective dose of INDO and EA offers a further benefit in that it may potentially decrease the side effects of drug therapy. Few studies have investigated the effect of EA–drug interaction in a well-controlled experimental condition. The present study provided an ideal behavioral animal model for such assessment.

The synergistic antihyperalgesic effect of the EA–INDO combination indicates that EA and INDO may act through different mechanisms to inhibit hyperalgesia. It is known that one mechanism of the analgesic effects of NSAIDs is the inhibition of cyclo-oxygenase-2 (COX-2; Vane, 1971; Abramson and Weissmann, 1989; Francischi et al., 2002). Major COX products at inflammatory sites are prostaglandins, which sensitize nociceptors (Cesare and McNaughton, 1997; Chen et al., 1999) and elicit hyperalgesia (Ferreira, 1980; Matsuzaki et al., 2002). EA's analgesic effect occurs via central release of endogenous opioids (Han, 2003), which exert inhibitory actions on excitatory transmissions in spinal dorsal horn neurons (Glaum et al., 1994; Stiller et al., 1993) and block the release of substance P, a nociceptive neuropeptide (Bourgoin et al., 1994). Studies showed that EA inhibits a tooth pulp stimulation-evoked release of immunoreactive SP (Yonehara et al., 1992) and reduces electrophysiological spinal neuron response to noxious stimuli (Yonehara et al., 1992). These data support our hypothesis that EA and INDO may act through different mechanisms, thus synergistically alleviating hyperalgesia. Previous studies showing that the coadministration of morphine and NSAID to rats (Lashbrook et al., 1999; Deciga-Campos et al., 2003) and humans (Reynolds et al., 2003) shows enhanced antinociception are also consistent with this hypothesis.

EA at 10 Hz showed a significant inhibitory effect on CFA-induced edema. This is consistent with previous

reports that acupuncture is effective in inhibiting inflammation in collagen arthritis (Fang et al., 1999) and carrageen-induced inflammation (Zhang et al., 2004) models. However, the combination of EA and INDO showed no addition or synergism, which indicates that EA and INDO may share the same pathways for suppressing inflammation. For example, the production and release of substance P, a mediator of inflammation, are inhibited by COX inhibitor (Ma and Eisenach, 2003) and EA (Yonehara et al., 1992), respectively. However, the detailed mechanism(s) underlying EA antiinflammation need to be further clarified.

In summary, the NSAIDs commonly used for the management of chronic pain (Katz, 2002; Immer et al., 2003) are accompanied by gastrointestinal disturbances and impairment of renal function (Scheiman, 2001; Immer et al., 2003). That the combination of a subeffective dose of INDO and EA treatment produces greater antihyperalgesia than either agent alone and than the sum effects of the individual agents suggests that EA is an effective adjunct to NSAIDs in the treatment of pain and may provide an improved treatment strategy for inflammatory pain.

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