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Research report

Additive anti-hyperalgesia of electroacupuncture and intrathecal antisense oligodeoxynucleotide to interleukin-1 receptor type I on carrageenan-induced inflammatory pain in rats

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ABSTRACT

Accumulating evidence shows that spinal interleukin-1β (IL-1β) plays a critical role in inflammatory pain. Electroacupuncture (EA) can effectively attenuate inflammatory hyperalgesia both in clinical practices and experimental studies. However, little is known about the relationship between spinal IL-1β and EA analgesia. The present study was designed to evaluate the effects of EA and antisense oligodeoxynucleotide (ODN) to IL-1 receptor type I (IL-1RI) on carrageenan-induced thermal hyperalgesia and the expression of IL-1β as well as IL-1RI. It was demonstrated that carrageenan induced marked thermal hyperalgesia in the injected paw, hence making paw withdrawal latency (PWL) decrease to 3.47 ± 0.31 s at 180 min post-injection. Nevertheless, when EA was administered for 30 min at 180 min post-carrageenan injection, the PWLs were significantly increased between 10 and 90 min following the beginning of EA treatment and peaked at 30 min to 5.91 ± 0.61 s. And also EA partly reversed the elevation of IL-1β and IL-1RI expression induced by carrageenan. Down-regulation of IL-1RI expression by repeated intrathecal antisense ODN (50 μg/10 μl) significantly increased the mean PWL up to 5.75 ± 0.15 s in 180–300 min post-carrageenan injection. Additionally, when the combination of EA with antisense ODN was used, thermal hyperalgesia was further alleviated than EA or antisense ODN alone, with a maximum PWL of 7.66 ± 0.50 s at 30 min post the beginning of EA treatment. The results suggested an involvement of the spinal IL-1β/IL-1RI system in EA-induced anti-hyperalgesia in inflammatory pain.

1. Introduction

Interleukin-1β (IL-1β, the predominant releasing form of IL-1) is a proinflammatory cytokine, which plays a major role in inflammation and immunity. Evidence shows that IL-1β is associated with the nociceptive modulation in the central nervous system and can be nociceptive or anti-nociceptive. Researchers have found that intracerebroventricular injection of IL-1β to the rats exerts biphasic responses on thermal and mechanical nociceptive thresholds depending upon the dosage, causing hyperalgesia at lower doses and analgesia at higher doses [8,23,24,27]. Intrathecal (i.t.) administration of IL-1β also shows varying nociceptive responses, and may promote the development of inflammatory and neuropathic pain [10,28,35,38,30,40] or reduce inflammatory pain [16,33]. However, the function of spinal endogenous IL-1β in inflammatory pain was still unclear, and therefore merits further investigation.

Two types of IL-1 receptor proteins have been reported to be identified. IL-1 signalling activity appears to be mediated exclusively via the IL-1 receptor type I (IL-1RI), whereas the IL-1 receptor type II (IL-1RII) has no signalling property and acts as a “decoy” target for IL-1, inhibiting its activity by preventing IL-1 from binding to the signalling IL-1RI [31]. By down-regulating the expression of IL-1RI, the biological activities of IL-1β can be better elucidated. Antisense oligodeoxynucleotide (ODN) strategy is a successfully used approach for many years. It has been reported that antisense ODN to IL-1RI can specifically down-regulate the expression of IL-1RI and inhibit the effect of IL-1β [2,5,12]. Nevertheless, it has rarely been used to study the role of the IL-1β/IL-1RI system in inflammatory pain.

Acupuncture, a traditional therapeutic modality from Traditional Chinese Medicine, has been used in China for thousands of

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years to treat a variety of diseases and symptoms with few side effects [7], which has been widely accepted by World Health Organization and other countries. Electrical stimulation of acupuncture points, which is called electroacupuncture (EA), is widely used both in clinic and in experimental studies. EA studies have also been performed on peripheral pathological pain animal models, such as carrageenan-induced inflammatory pain [29,39,6,18]. It has been reported that peripheral IL-1β was involved in EA analgesia during hyperalgesia [29]. However, the mechanism of spinal IL-1β in EA analgesia has not been fully understood.

Thus, the aim of the present study, using the carrageenan-induced inflammatory pain model in the rats, was to determine (1) whether inhibition of IL-1RI expression blocks thermal hyperalgesia; (2) whether EA modulates the expression of IL-1β and IL-1RI; (3) whether down-regulation of IL-1RI expression enhances the anti-hyperalgesia of EA.

2. Materials and methods

2.1. Rats

The experiments were performed on adult male Sprague–Dawley rats (Experimental Animal Center, Shanghai Medical College of Fudan University, China) weighing 200–220 g, which were allowed to acclimate for 1 week and maintained on a 12:12-h light-dark cycle with free access to food and water prior to experimental manipulation. All the experiments were carried out in the light cycle between 08:00 and 16:00 to avoid diurnal variation. The animal protocols were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and the guidelines of the International Association for the Study of Pain [41]. All efforts were made to minimize the number of animals used and their suffering.

2.2. Carrageenan inflammation

Inflammatory pain was induced by intraplantar (i.pl.) injection of carrageenan (λ-carrageenan, Sigma, 2 μg/100 μl of normal saline (0.9% NaCl)) into unilateral hind paw of non-anesthetized rats according to the previous method [37]. The contralateral paw was untreated; the inflammation, which appeared shortly after injection in the form of redness, edema and hyper-responsiveness to noxious stimuli was limited to the injected paw and lasted about 72 h. The rats were used to perform the behavioral test at 3 h following the injection of carrageenan, corresponding to the peak inflammatory response.

2.3. EA administration

EA was administered at 3 h post-carrageenan injection. The detailed EA procedure has been described previously [39]. In brief, during EA treatment, the trunk was kept motionless, and a pair of stainless steel needles of 0.3 mm diameter were inserted with a depth of 5 and 3 mm, respectively into the unilateral acupuncture points (ipsilateral to carrageenan-injected paw) “Zu San Li” (ST36, located near the knee joint, between the muscle anterior tibialis and muscle extensor digitorum longus) and “Kun Lun” (UB60, located near the ankle joint, between the tip of the external malleolus and tendo calcaneus). The two pins were connected with the output terminals of an EA apparatus (Model G-6805-1A, Shanghai Huayi Medical Electronic Apparatus Company, China). Alternating trains of dense-sparse frequencies (60 Hz for 1.05 s and 2 Hz for 2.85 s alternately) were selected, with the intensity of stimulation increased according to a preset schedule of 1–2–3 mA, and each lasting for 10 min [39]. In order to exclude the stress-induced analgesia by animal fixation and needle insertion, the group treated with sham EA underwent the same manipulation as one with EA except electrical stimulation. This form of sham EA showed little anti-hyperalgesia [17] and seemed to be an appropriate control for non-specific needling effect.

2.4. Intrathecal administration

Chronically indwelling i.t. catheters were implanted into the subarachnoid space of lumbar enlargement of the rats according to the previous method [34] for ODN administration. Briefly, an i.t. catheter (PE-10 tube) was inserted through the gap between the L4 and L5 vertebrae and extended to the subarachnoid space of the lumbar enlargement (L4 and L5 segments) under sodium pentobarbital (40 mg/kg) anesthesia by intraperitoneal (i.p.) injection. The catheter was filled with sterile normal saline (approximately 4 μl), and the outer end was plugged. The external end of the tube was passed subdermally and secured to the back of the neck where an incision had been made to allow exit. The animals were allowed to recover from the implantation surgery for 3 days prior to any experiment, and monitored daily after surgery for signs of motor deficiency. Those that showed any neurological deficits resulting from the surgical procedure were excluded from the experiments. The location of the distal end of the i.t. catheter was verified at the end of every experiment by injection of Pontamine Sky Blue via the i.t. catheter.

2.5. Antisense ODN

Down-regulation of IL-1RI was ensured via i.t. delivery of antisense ODN specifically complementary to a segment of the sequence of IL-1RI mRNA. The sequence of antisense ODN was: 5′-CACTTTCATATTCTCCAT-3′. The sense ODN to IL-1RI (5′-ATGGAGAATATGAAAGTG-3′) was used as the control. These two kinds of ODNs were fully phosphorothioated, with the antisense ODN sequence, proved to be effective and specific previously [5,12], based on the rat IL-1RI sequence obtained from neural tissue [14]. They were used at a dose of 50 μg dissolved in 10 μl of nuclease-free normal saline per injection per rat, and each i.t. injection of ODN was followed by 5 μl normal saline flush, once daily for 3 days as a pretreatment before carrageenan injection.

2.6. Behavioral test

The rats were tested for hind paw thermal hyperalgesia using a method developed previously [13]. Briefly, they were placed under a clear plastic chamber on the glass surface of the Model 390 paw stimulator analgesia meter (IITC/Life Science Instruments, USA) and allowed to acclimatize for 30 min. The radiant light focused onto the plantar surface of each hind paw. The duration from the onset of radiant heat application to the hind paw’s withdrawal was defined as the paw withdrawal latency (PWL), a measure of thermal hyperalgesia. Both hind paws were tested independently with a 10-min interval between trials, with the intensity of the thermal stimulus adjusted to derive an average baseline PWL of approximately 8–10 s in naive animals, and a cut-off time of 20 s for stimulation designed to prevent tissue damage. Baseline PWL was measured before any i.t. injections or EA treatment and averaged from 4 PWLs for one experiment, and the investigators were blind to the experimental condition of each rat.

2.7. ELISA

Concentration of IL-1β in the spinal cord was detected by ELISA. At 3.5 h post-carrageenan injection, the rats were sacrificed with an overdose of urethane (1.5 g/kg, i.p.) and the L4–L6 segments of the spinal cord were collected in dry ice and stored at −70 °C until the time of sonication. Total protein was dissociated mechanically from tissue using an ultrasonic cell disruptor, and then centrifuge sonicated at 10,000 × *g* at 4 °C for 10 min. And the supernatant was removed and stored at −70 °C until an ELISA was performed.

IL-1β ELISA kit was from R&D Systems. Ninety-six flat-bottom wells were coated with sheep anti-rat IL-1β immunoaffinity-purified polyclonal antibody overnight at 4 °C, and washed in assay buffer. To each well were added 100 μl of rat IL-1β standards or samples, which were incubated at room temperature for 4 h. Following washing in assay buffer, 100 μl of biotinylated, immunoaffinity-purified polyclonal sheep anti-rat IL-1β antibody (1:2000) with 1% normal goat serum was added to each well of the plates, which were incubated at room temperature for 1 h. The color was developed using avidin-horseradish peroxidase (HRP) and the chromogen orthophenylene diamine (Sigma). The plates were read at 490 nm, and data were normalized to total protein levels as determined using the BCA-Assay (Pierce, Rockville, IL, USA) and expressed as pg cytokine/100 μg total protein.

2.8. RT-PCR

To examine the changes of mRNA level of IL-1RI, RT-PCR was performed according to the following protocol. At 3.5 h post-carrageenan injection, the rats were sacrificed with an overdose of urethane (1.5 g/kg, i.p.) and the L4–L6 segments of the spinal cord were collected in dry ice. Total RNA extraction was performed using the Trizol reagent, following the instructions of the manufacturer. RNA was further purified using the RNeasy kit in accordance with the RNA clean-up protocol, and eluted in 50 μl of RNase-free distilled H_2O. The amount of RNA was measured spectrophotometrically. One μg of total RNA was applied to the synthesis of the first strand of cDNA via the SuperScript × reverse transcriptase. Briefly, RNA, oligo (dT) 18 primers (0.5 μg/μl) were first denatured for 5 min at 65 °C, chilled on ice for 1 min, and then incubated for 50 min at 42 °C, 15 min at 70 °C in 20 μl of a reaction mixture containing 10× first-strand buffer, 10 mM dNTP mix, 0.1 M DTT and 50 units of SuperScript II reverse transcriptase. The sequences of primers were as follows: IL-1RI forward, 5′-ACACATGGTATAGATGCAGC-3′; reverse, 5′-AGAACGGACTCCAGAACCTT-3′ [1]; β-actin forward, 5′-CACCATGTACCCTGGCATTG-3′; reverse, 5′-TAACGCAACTAAGTCATAGT-3′. The primers were synthesized and purified by Sangon Biotech Co. Ltd. (Shanghai, China). One microlitre of cDNA was added to 49 μl of PCR mix containing 5× PCR buffer, 18 pmol/l concentrations of each primer, 2.5 mM of dNTP, and three units of Pfu DNA polymerase. PCR reaction was performed as follows: 12 min at 94 °C to activate the Taq polymerase, followed by 28 cycles of 1 min at 94 °C, annealing 50 °C for 1 min and extension 72 °C for 1 min. A final elongation step at 72 °C for 10 min completed the PCR reaction. Ten microlitres of each PCR production was electrophoresed in 2% agarose gel, visualized by ethidium bromide staining and scanned with ultraviolet transilluminator (GDS 8000, Gene Tools from Syngene software, U.K.). The PCR quantitative method took advantage of the fact that β-actin was employed as internal standard under the same condition. All the results were expressed as ratios of the intensity of the IL-1RI bands to that of β-actin band.

2.9. Western blot

Western blot was performed to examine the changes of the expression of IL-1RI. At 3.5 h post carrageenan injection, the rats were sacrificed with an overdose of urethane (1.5 g/kg, i.p.) to collect L4–L6 segments of the spinal cord on dry ice, which were to be stored at −70 °C until assayed. Each sample was weighed and homogenized in 1.5 ml of sample buffer (0.01 M Tris–HCl buffer (pH 7.6) containing 0.25 M sucrose, 0.1 M NaCl, 1 mM EDTA, and 1 mM phenylmethylsulfonylfluoride) at 4 °C. Following 12,000 r.p.m. centrifugation at 10 min supernatant was applied for Western blotting. The samples (30 μg of total protein) were dissolved with equal volume of loading buffer (0.1 M Tris–HCl buffer (pH 6.8) containing 0.2 M DTT, 4% SDS, 20% glycerol and 0.1% bromophenol blue), separated on 10% SDS–PAGE and then electrotransferred at 100 V to Immun-Blot PVDF membrane for 1 h at 4 °C, and the membranes were blocked in TBST containing 5% non-fat dried milk overnight at 4 °C prior to incubation for 2 h at room temperature with anti-IL-1RI polyclonal antibody (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA) diluted in TBST containing 5% BSA. Blots washed extensively in TBST and incubated with goat anti-rabbit IgG conjugated to HRP (Santa Cruz) in TBST/1.25% BSA for 1 h at room temperature, the signal was detected by an enhanced chemiluminescence method (ECL kit, Santa Cruz), and exposed to Kodak X-OMAT film (Eastman Kodak, Rochester, NY, U.S.A.), and the intensity of the bands was captured and analyzed using GeneSnap Image Analysis Software (Syngene, U.K.).

2.10. Statistical analysis

All data were presented as mean ± S.E.M. and analyzed by SPSS 11.5, and repeated measures analysis of variance (ANOVA) was conducted for overall effects, with the Student–Newman–Keul test for post hoc analysis for differences between groups. The ELISA, RT-PCR and western blot data were analyzed by ordinary ANOVA. $P < 0.05$ was considered statistically significant.

3. Results

3.1. EA alleviating thermal hyperalgesia in carrageenan inflammatory pain

The effects of carrageenan on the PWL of both hind paws were observed first. The baseline PWLs for carrageenan- and non-injected paws were 9.35 ± 0.63 and 9.32 ± 0.52 s, respectively ($n = 7$, Fig. 1). I.pl. injection of carrageenan (2 μg/100 μl) produced marked inflammation (edema and erythema) and thermal hyperalgesia in the injected paw ($P < 0.01$ to $P < 0.001$), which made the PWL decrease, comparing to the non-injected control, to 3.47 ± 0.31 s at 180 min post injection, and presented little change in magnitude for the next 120 min.

At 180 min following carrageenan injection, EA stimulation applied to the ipsilateral (carrageenan-injected) paw at the 'ST36' and 'UB60' acupuncture points significantly reduced thermal hyperalgesia induced by carrageenan ($P < 0.05$ to $P < 0.001$, $n = 9$–11, Fig. 2). The ipsilateral PWLs of the carrageenan plus EA group were significantly increased between 10 and 90 min post the beginning of EA treatment and peaked at 30 min to 5.91 ± 0.61 s. However, the contralateral PWLs showed no obvious change (data not shown). As a control, sham EA demonstrated no effect on the PWLs. These data indicated that EA significantly reduced inflammation-induced thermal hyperalgesia.

3.2. EA reducing carrageenan-induced IL-1β expression in the spinal cord

To investigate the relationship between EA analgesia and IL-1β, the expression of IL-1β in L4–L6 segments of the rats' spinal cord was examined by using ELISA. The protein level of IL-1β was detected to be 1 ± 0.22 pg per 100 μg total protein in the normal

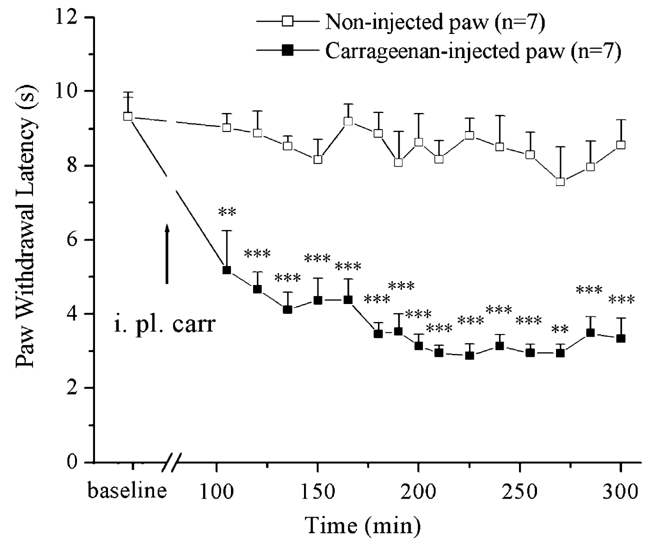

Fig. 1. Thermal hyperalgesia induced by carrageenan on the PWLs of carrageenan-injected and non-injected paws. Baseline was measured prior to carrageenan injection (i.pl. carr), and the data were expressed as mean ± S.E.M. **$P < 0.01$ and ***$P < 0.001$ *vs.* non-injected paw group.

group, while at 3.5 h following a carrageenan shot, it was remarkably raised to 13.6 ± 1.21 pg per 100 μg total protein ($P < 0.001$, $n = 6$, Fig. 3). EA significantly reduced carrageenan-induced expression of IL-1β by 40.1 ± 7.9% ($P < 0.05$). There was no significant difference between the group treated with carrageenan plus sham EA and one with carrageenan ($P > 0.05$). The results implicated that IL-1β in the spinal cord was involved in carrageenan-induced inflammation and EA analgesia.

3.3. EA reducing carrageenan-induced IL-1RI expression in the spinal cord

To demonstrate the effect of EA on IL-1RI expression, L4–L6 segments of the spinal cord from the four groups were removed and assayed by RT-PCR and Western blot analysis, respectively.

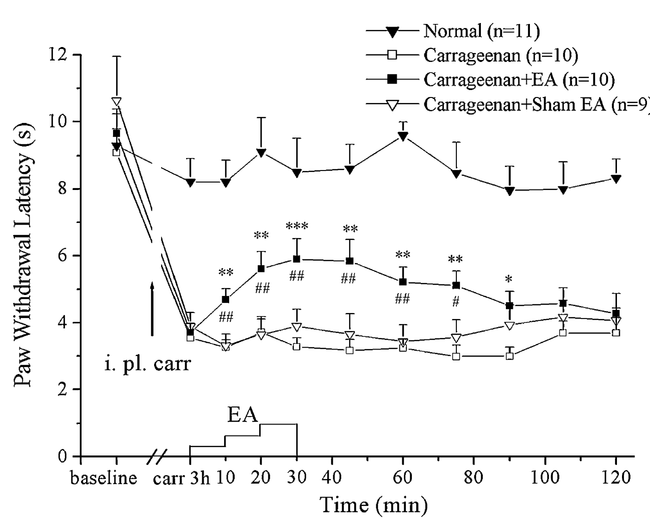

Fig. 2. Effects of EA on the PWLs of carrageenan-injected paw. Baseline was measured before carrageenan injection (i.pl. carr). EA was administered 3 h following the injection of carrageenan, and lasting for 30 min. The data were expressed as mean ± S.E.M. Carr3 h: 3 h post-carrageenan injection. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ *vs.* carrageenan group; #$P < 0.05$ and ##$P < 0.01$ *vs.* Carrageenan plus sham EA group.

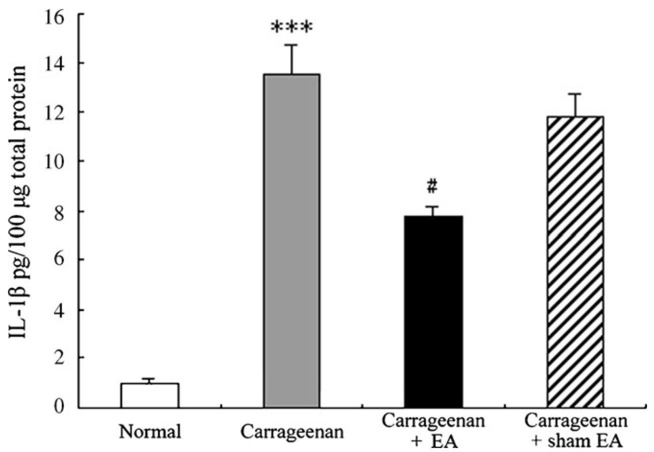

Fig. 3. Effects of EA on IL-1β expression induced by carrageenan in the spinal cord. EA was administered at 3 h post-carrageenan injection, and lasting for 30 min. L4–L6 segments of the spinal cord were removed 3.5 h post-carrageenan injection and assayed by ELISA. The data were expressed as mean ± S.E.M., $n = 6$. ***$P < 0.001$ *vs.* normal group; #$P < 0.05$ *vs.* carrageenan group.

RT-PCR analysis obtained an expected 300-bp product for IL-1RI mRNA (Fig. 4A); the semi-quantitive analysis showed a significant change that at 3.5 h post-carrageenan injection, the mRNA level of IL-1RI in the spinal cord was raised compared with that of the normal group ($P < 0.001$), and EA reduced carrageenan-induced mRNA expression of IL-1RI by 42.7 ± 5.7% ($P < 0.001$, $n = 6$, Fig. 4B). A single protein band of the expected size (~80 kDa) for IL-1RI was detected by Western blot with the IL-1RI-specific primary antibody (Fig. 4C). In addition, no band was detected when the primary antibody was omitted (data not shown). Density analysis of Western blot obtained a similar change with RT-PCR, i.e., EA resulted in a 53.2 ± 3.5% reduction of IL-1RI protein compared with the carrageenan group ($P < 0.01$, $n = 6$, Fig. 4D). However, the mRNA or protein level of IL-1RI was not influenced by sham EA ($P > 0.05$). This experiment suggested that IL-1RI in the spinal cord was also involved in carrageenan-induced inflammation and EA analgesia.

3.4. *Intrathecal antisense ODN down-regulating spinal IL-1RI expression*

To verify the effect of antisense ODN, the expression of IL-1RI in L4-L6 segments of the spinal cord was detected following i.t. delivery of ODNs (50 μg/10 μl) or normal saline (10 μl) once daily for 3 days. The semi-quantitive analysis of RT-PCR show a significant change that antisense ODN resulted in a 61.3 ± 3.6% and 50.3 ± 9.2% reduction of IL-1RI mRNA as compared with the control groups of normal saline ($P < 0.01$) and sense ODN treatment ($P < 0.05$, $n = 6$, Fig. 5A and B), respectively; Western blot analysis displayed a similar change that the protein expression of IL-1RI in the spinal cord of the antisense group was significantly inhibited by 65.8 ± 6.5% and 69.1 ± 6.7% as compared with the saline group and sense one, respectively ($P < 0.001$, $n = 6$, Fig. 5C and D). The resulting data conformably indicated that the expression of IL-1RI in the spinal cord could be significantly down-regulated by antisense ODN treatment.

3.5. *Antisense ODN to IL-1RI alleviating thermal hyperalgesia in carrageenan inflammatory pain*

To further assess the effect of spinal IL-1β and IL-1RI on carrageenan inflammatory pain, antisense ODN to IL-1RI was used to down-regulate the expression of IL-1RI. I.t. injection of ODNs or normal saline for 3 days to the normal rats led to

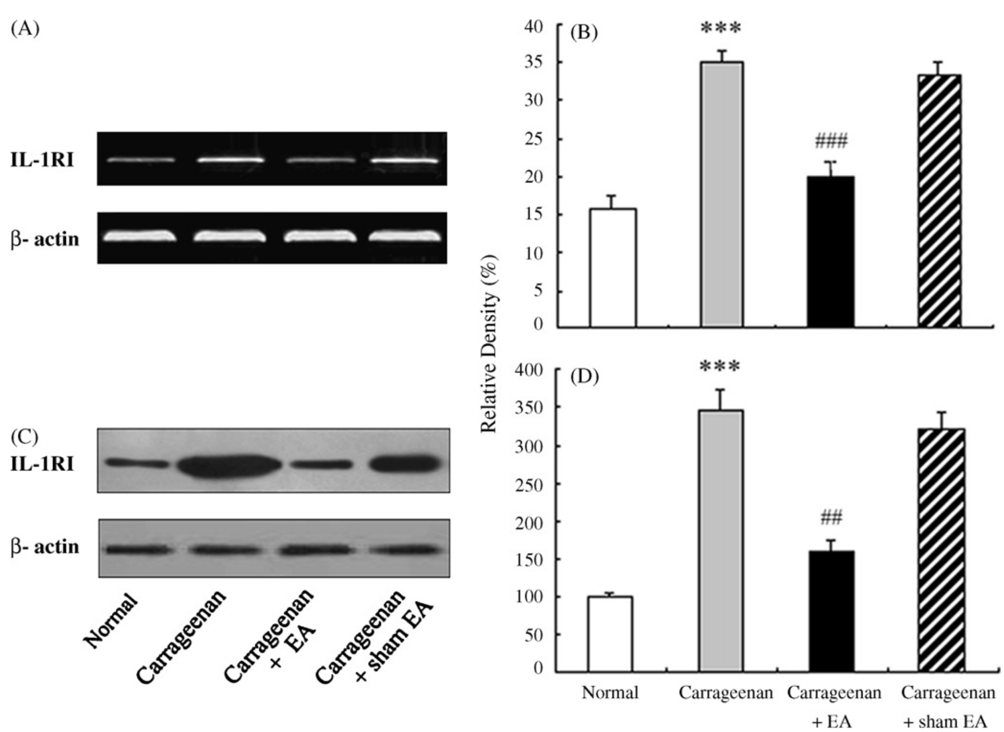

Fig. 4. Effects of EA on the expression of IL-1RI induced by carrageenan in the spinal cord. The PCR products of expected size were acquired corresponding to IL-1RI (A). Western blot analysis detected a protein band for IL-1RI (C). β-Actin and IL-1RI were produced from the same blot. The data were quantified and demonstrated. The mRNA levels of different groups were expressed as a percentage to that of corresponding β-actin (B). The optical densities of immunoblot bands were expressed as a percentage to that of the normal group sample (100%) (D). The data were expressed as mean ± S.E.M., $n = 6$. ***$P < 0.001$ *vs.* normal group; ##$P < 0.01$ and ###$P < 0.001$ *vs.* carrageenan group.

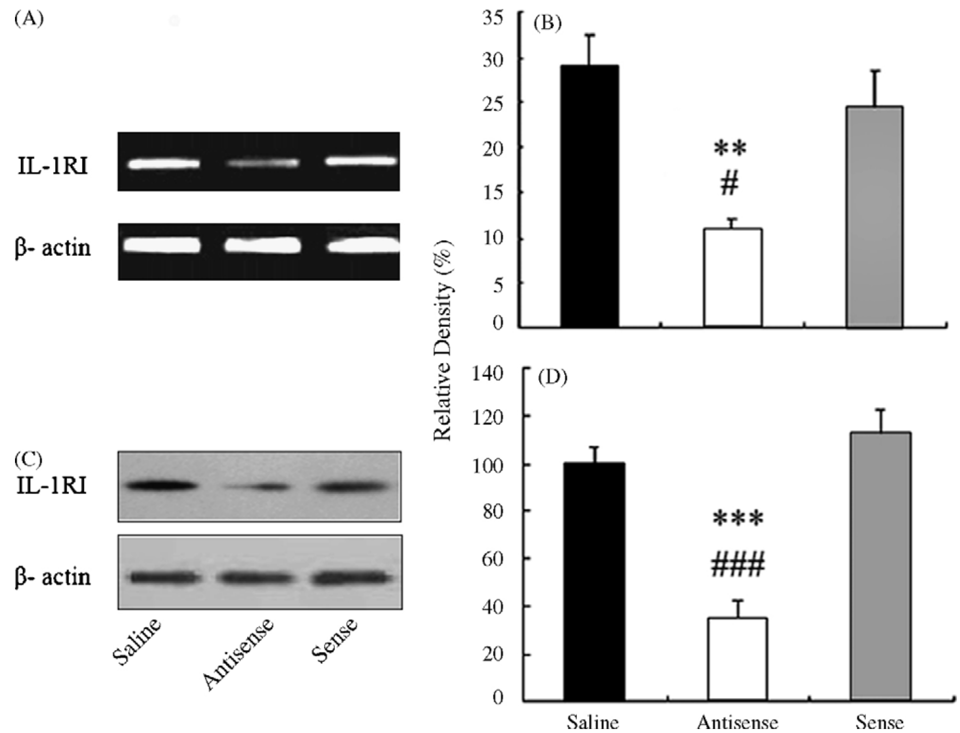

Fig. 5. Down-regulation IL-1RI expression by i.t. delivery of antisense ODN. The PCR products of expected size were acquired corresponding to IL-1RI (A). Western blot analysis detected a protein band for IL-1RI (C). The results were quantified and demonstrated. The mRNA levels of the different groups were expressed as a percentage to that of corresponding β-actin (B). The optical densities of immunoblot bands were expressed as a percentage to that of the saline group sample (100%) (D). The data were expressed as mean ± S.E.M., $n = 6$. $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs.* saline group; $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ *vs.* sense group.

no significant changes in the PWLs during the period (data not shown). Carrageenan was i.pl. injected on the 3rd day following i.t. injection, which consequently decreased the ipsilateral PWLs of all the animals ($n = 9$–10, Fig. 6). However, the rats of the antisense group showed lower hyperalgesia, with a mean PWL of 5.75 ± 0.15 s in 180–300 min post-carrageenan injection ($P < 0.05$ to $P < 0.001$). There was no obvious difference between the sense group and the saline group ($P > 0.05$), and the contralateral PWLs showed no obvious change (data not shown). The results indicated that down-regulating IL-1RI expression could significantly reduce inflammation-induced thermal hyperalgesia.

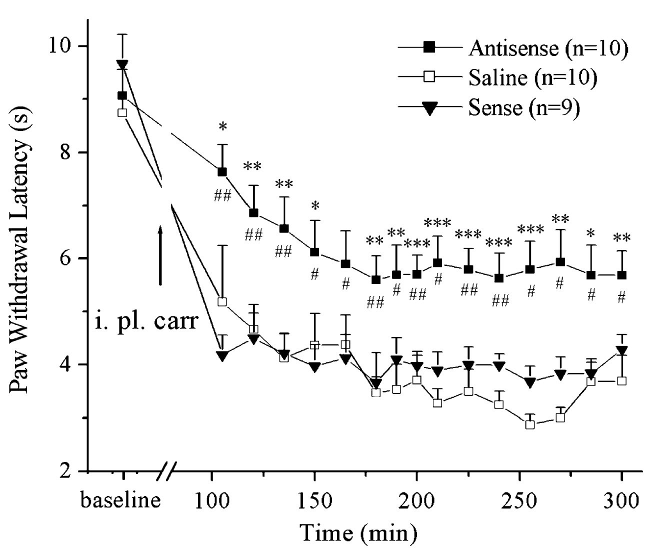

Fig. 6. Effects of antisense ODN to IL-1RI on the PWLs of carrageenan-injected paw. Antisense ODN (50 μg/10 μl), sense ODN (50 μg/10 μl) and normal saline (10 μl) were i.t. injected respectively, once daily for 3 days. On the 3rd day following i.t. injection, carrageenan was i.pl. injected. Baseline was measured post-i.t. injection and before carrageenan injection (i.pl. carr). The data were expressed as mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs.* saline group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs.* sense group.

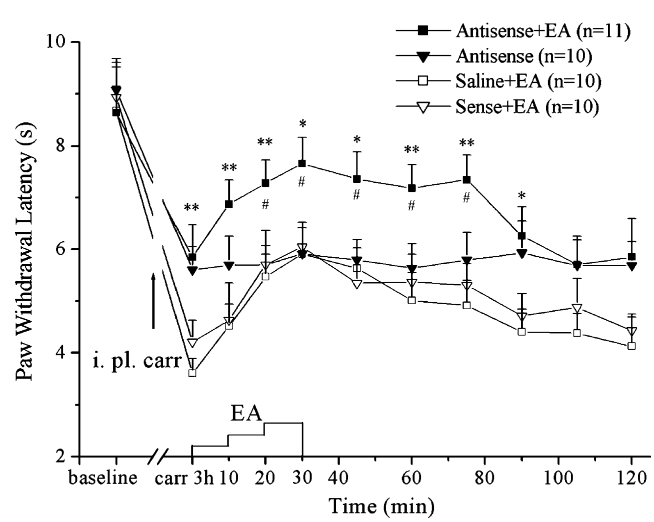

Fig. 7. Effects of antisense ODN to IL-1RI combined with EA on the PWLs of carrageenan-injected paw. Antisense ODN was delivered at a dose of 50 μg per injection (once daily) for 3 days. On the 3rd day following i.t. injection, carrageenan was i.pl. injected. Baseline was measured post-i.t. injection and before carrageenan injection (i.pl. carr). EA was administered at 3 h post-carrageenan injection, and lasting for 30 min. Carr3 h: 3 h post-carrageenan injection. The data were expressed as mean ± S.E.M. $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs.* saline plus EA group; $^{\#}P < 0.05$ *vs.* antisense group.

3.6. Antisense ODN to IL-1RI combined with EA additively inhibiting thermal hyperalgesia

To clarify whether endogenous IL-1β is related to EA analgesia, EA was administered following i.t. injections of antisense ODN for 3 days. As compared with the group of antisense, saline plus EA or sense plus EA, the one with antisense plus EA presented significant higher PWLs of carrageenan-injected paw ($P < 0.05$ to $P < 0.01$, $n = 10–11$, Fig. 7), which reached a maximum of 7.66 ± 0.50 s at 30 min post the beginning of EA treatment. Few significant differences were observed between the groups treated with saline plus EA and one with sense plus EA. The contralateral PWLs showed no obvious change (data not shown). The results suggested that carrageenan-induced thermal hyperalgesia was significantly lower when EA was combined with antisense ODN than when EA or intrathecally injection of antisense ODN was used alone.

4. Discussion

4.1. Antisense ODN to IL-1RI alleviating carrageenan-induced pain hypersensitivity

In clinical practice, inflammatory pain is one of the most common types of pathological pain. In current pain modulation research, carrageenan injection into the rat's hind-paw provides a convenient animal model that has been widely used to study the mechanism of nociception. This model is characterized by both a rapid onset and resolution of inflammation that causes a restricted distribution of hyperalgesia. Indeed, maximum hyperalgesia occurs 3–4 h post injection, and the hyperalgesic inflammation is resolved substantially by 24–74 h [13,25].

To investigate the role of IL-1β, IL-1 receptor antagonist (IL-1Ra) has been used as a competitive inhibitor binding to IL-1 receptors [3]. Although its affinity to IL-1RII is low, IL-1Ra may not accurately discriminate between IL-1 receptor subtypes. However, being the decoy receptor of IL-1β, IL-1RII may not play an entirely passive role, and was reported to be involved in IL-1 inhibition and constitute an important negative feedback mechanism [9]. Inhibitory activity of IL-1RII has been shown to increase the concentration of IL-1β in the medium of lipopolysaccharide-treated microglia [26]. Moreover, the expression of IL-1RII on neutrophils could internalize IL-1β [4]. Therefore, the function of IL-1/IL-1RI system may not be well clarified using IL-1Ra. To exclude the disturbance of IL-1RII, antisense ODN to IL-1RI was used in the current work. Antisense ODN is an attractive potential approach to down-regulating the expression of cell-surface receptors and attenuating cellular responsiveness, predominantly thanks to its nonpeptidic nature and potential to be absolutely specific [5]. Of great interest, therefore, was the observation that antisense ODN to IL-1RI inhibited IL-1 stimulated prostaglandin E2 synthesis and the infiltration of neutrophils on cultured cells, and when intracerebroventricular administered, inhibited the anorexia induced by IL-1β [5,12]. In the present study, antisense ODN to IL-1RI was also proved to be effective in down-regulating the expression of IL-1RI in the spinal cord, suggesting that it could be applied to effectively investigate the role of the IL-1β/IL-1RI system.

We observed that i.t. delivery of antisense ODN performed a significant anti-hyperalgesic effect in the rats with inflammatory pain, suggesting that endogenous IL-1β in the spinal cord participated in carrageenan-induced thermal hyperalgesia. The findings were consistent with those of previous investigations. Whether i.t. injection of IL-1β (at the dose of 50 pg to 100 ng) was administered or its function was blockaded, IL-1β was shown to be associated with the development of inflammatory hyperalgesia, and play a role in creating exaggerated pain states on inflammatory animals [10,28,35,15,30]. However, it was reported that i.t. delivery of IL-1β (at the dose of 10–100 ng) exerted a significant anti-nociceptive effect [16,33]. The reason why there are conflicting results is still unclear, and therefore warrants further investigations. A possible reason for the differences between these evidences may be that spinal IL-1β performs different functions depending on its concentration: the endogenous and lower doses of exogenous IL-1β enhanced hyperalgesia, mediated by induction of cyclooxygenase-2 and prostaglandin E2 synthesis [28], and the higher doses of exogenous IL-1β produced analgesia through opioid or 5-hydroxytryptamine systems [16]. In addition, different models of inflammation and differences in experimental conditions, such as the weight of rats, the source of reagents, and the methods used to test nociception, may have been involved.

Our study indicated that antisense ODN to IL-1RI exercised no effect on the PWLs when injected intrathecally into normal, uninflamed rats. Considering the previous reports that i.t. injection of IL-1Ra was ineffective in altering basal pain sensitivity [20,36], we postulated that IL-1β might not alter physiological protective pain which was induced by noxious radiant heat stimulus and elicited paw withdrawal responses.

4.2. EA reversing the elevation of IL-1β and IL-1RI expression induced by carrageenan in the spinal cord

EA has been widely used in both clinical practice in acupuncture treatment and experimental research because it is manageable and easy to quantify, and thus repeatable. For sham EA control, acupuncture needles were also inserted into acupoints as one of the most commonly used control treatments, but without electrical current. This sham procedure produced little anti-hyperalgesia and was widely employed in many studies [39,17,21]. In the present study, ST36 and UB60 were chosen on Traditional Chinese Medicine meridian theory [22], and its successful use in our previous studies [39], showing significant anti-hyperalgesia. Our data demonstrated again that EA at ST36 and UB60 could significantly increase the PWLs of the carrageenan-injected rats, suggesting an analgesic effect in this model.

Being a key pro-inflammatory cytokine, IL-1β has been reported having some relationship with EA analgesia. Previous work demonstrated that EA could reduce inflammation-induced IL-1β expression. EA stimulation significantly inhibited the concentrations of endogenous IL-1β in splenocytes and synovial tissues of type II collagen-induced arthritic mice [11]. The hypothalamic and ventral midbrains mRNA levels of IL-1β raised by inflammation could be reversed to normal levels by acupuncture stimulation [19,32]. Additionally, EA could markedly reduce monoarthritis-induced up-regulation of spinal IL-1β [21]. In the present study, we also found EA reduced inflammation-induced IL-1β expression in the spinal cord. Furthermore, spinal IL-1RI expression induced by inflammation was reversed as well. Associating the results mentioned above that spinal IL-1β and IL-1RI participated in thermal hyperalgesia, we hypothesized that EA might act its analgesic effect partly via reducing the expression of the IL-1β/IL-1RI system.

4.3. Additive anti-hyperalgesia of EA and intrathecal ODN to IL-1RI

In the present work, it was observed that EA and antisense ODN additively inhibited carrageenan-induced thermal hyperalgesia. This might be due to the reduction of IL-1β and IL-1RI by EA and antisense ODN, respectively. Our study showed that EA could reduce carrageenan-induced expression of IL-1β by $40.1 \pm 7.9\%$ (see Fig. 3). However, antisense ODN was observed no effect on the expression of IL-1β. The group treated with antisense plus EA presented a lower level of IL-1β than one with antisense, but a similar level with saline plus EA or sense plus EA group (data not shown). On the other hand, although EA resulted in a $53.2 \pm 3.5\%$ reduction of IL-1RI protein as compared with the carrageenan group, the IL-1RI expression of EA group was still higher than that of the normal group (see Fig. 4). However, antisense ODN could significantly inhibit the expression of IL-1RI protein by $65.8 \pm 6.5\%$ as compared with the saline group at the normal status (see Fig. 5). Additionally, in carrageenan-induced inflammation, the group treated with antisense plus EA showed more effective inhibition on the expression of IL-1RI than one with EA, but without a significant difference from one with antisense (data not shown). Thus, with lower level of IL-1β than antisense ODN alone and IL-1RI than EA alone, the combination of EA with antisense ODN could additively inhibit carrageenan-induced hyperalgesia.

Conflict of interest

The authors declare that they have no competing financial interests.

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References

[1] M. Abdul, N. Hoosein, Relationship of the interleukin-1 system with neuroendocrine and exocrine markers in human colon cancer cell lines, Cytokine 18 (2002) 86–91.
[2] S. Akhtar, S. Agrawal, In vivo studies with antisense oligonucleotides, Trends Pharmacol. Sci. 18 (1997) 12–18.
[3] W.P. Arend, Interleukin-1 receptor antagonist, Adv. Immunol. 54 (1993) 167–227.
[4] E. Bourke, A. Cassetti, A. Villa, E. Fadlon, F. Colotta, A. Mantovani, IL-1 beta scavenging by the type II IL-1 decoy receptor in human neutrophils, J. Immunol. 170 (2003) 5999–6005.
[5] R.M. Burch, L.C. Mahan, Oligonucleotides antisense to the interleukin 1 receptor mRNA block the effects of interleukin 1 in cultured murine and human fibroblasts and in mice, J. Clin. Invest. 88 (1991) 1190–1196.
[6] Y. Chae, M.S. Hong, G.H. Kim, D.H. Hahm, H.J. Park, E. Ha, M.J. Kim, H.J. Park, J. Yang, H. Lee, Protein array analysis of cytokine levels on the action of acupuncture in carrageenan-induced inflammation, Neurol. Res. 29 (2007) S55–58.
[7] X. Cheng, Chinese Acupuncture and Moxibustion, Foreign Languages Press, Beijing, 1999, 590 pp.
[8] A. Coelho, J. Fioramonti, L. Bueno, Brain interleukin-1β and tumor necrosis factor-alpha are involved in lipopolysaccharide-induced delayed rectal allodynia in awake rats, Brain Res. Bull. 52 (2000) 223–228.
[9] F. Docagne, S.J. Campbell, A.F. Bristow, S. Poole, S. Vigues, C. Guaza, V.H. Perry, D.C. Anthony, Differential regulation of type I and type II interleukin-1 receptors in focal brain inflammation, Eur. J. Neurosci. 21 (2005) 1205–1214.
[10] M. Falchi, F. Ferrara, C. Gharib, B. Dib, Hyperalgesic effect of intrathecally administered interleukin-1 in rats, Drugs Exp. Clin. Res. 27 (2001) 97–101.
[11] J.Q. Fang, E. Aoki, Y. Yu, T. Sohma, T. Kasahara, T. Hisamitsu, Inhibitory effect of electroacupuncture on murine collagen arthritis and its possible mechanisms, In Vivo 13 (1999) 311–318.
[12] S. Gayatri, C.F. Mark, R.P. Carlos, Interleukin-1 receptor type I mediates anorexia but not adipsia induced by centrally administered IL-1β, Physiol. Behav. 62 (1997) 1179–1183.
[13] K. Hargreaves, R. Dubner, F. Brown, C. Flores, J. Joris, A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia, Pain 32 (1988) 77–88.
[14] R.P. Hart, C. Liu, A.M. Shadiack, R.J. McCormack, G.M. Jonakait, An mRNA homologous to interleukin-1 receptor type I is expressed in cultured rat sympathetic ganglia, J. Neuroimmunol. 44 (1993) 49–56.
[15] P. Honore, C.L. Wade, C. Zhong, R.R. Harris, C. Wu, T. Ghayur, Y. Iwakura, M.W. Decker, C. Faltynek, J. Sullivan, M.F. Jarvis, Interleukin-1alphabeta gene-deficient mice show reduced nociceptive sensitivity in models of inflammatory and neuropathic pain but not post-operative pain, Behav. Brain Res. 167 (2006) 355–364.
[16] G.C. Ji, Y.Q. Zhang, F. Ma, G.C. Wu, Increase of nociceptive threshold induced by intrathecal injection of interleukin-1beta in normal and carrageenan inflammatory rat, Cytokine 19 (2002) 31–36.
[17] L.X. Lao, R.X. Zhang, G. Zhang, X. Wang, B.M. Berman, K. Ren, A parametric study of electroacupuncture on persistent hyperalgesia and Fos protein expression in rats, Brain Res. 1020 (2004) 18–29.
[18] J.H. Lee, K.J. Jang, Y.T. Lee, Y.H. Choi, B.T. Choi, Electroacupuncture inhibits inflammatory edema and hyperalgesia through regulation of cyclooxygenase synthesis in both peripheral and central nociceptive sites, Am. J. Chin. Med. 34 (2006) 981–988.
[19] X.Y. Liu, H.F. Zhou, Y.L. Pan, X.B. Liang, D.B. Niu, B. Xue, F.Q. Li, Q.H. He, X.H. Wang, X.M. Wang, Electro-acupuncture stimulation protects dopaminergic neurons from inflammation-mediated damage in medial forebrain bundle-transected rats, Exp. Neurol. 189 (2004) 189–196.
[20] S.F. Maier, E.P. Wiertelak, D. Martin, L.R. Watkins, Interleukin-1 mediates the behavioral hyperalgesia produced by lithium chloride and endotoxin, Brain Res. 623 (1993) 321–324.
[21] W.L. Mi, Q.L. Mao-Ying, Q. Liu, X.W. Wang, Y.Q. Wang, G.C. Wu, Synergistic antihyperalgesia of electroacupuncture and low dose of celecoxib in monoarthritic rats: involvement of the cyclooxygenase activity in the spinal cord, Brain Res. Bull. 77 (2008) 98–104.
[22] J. O'Connor, D. Bensky, Acupuncture:, A Comprehensive Text, Eastland Press, Chicago, 1981, 741 pp.
[23] T. Oka, S. Aou, T. Hori, Intracerebroventricular injection of interleukin-1 beta induces hyperalgesia in rats, Brain Res. 624 (1993) 61–68.
[24] T. Oka, S. Aou, T. Hori, Intracerebroventricular injection of interleukin-1β enhances nociceptive neuronal responses of the trigeminal nucleus caudalis in rats, Brain Res. 656 (1994) 236–244.
[25] A. Pertovaara, M.M. Hamalainen, T. Kauppila, P. Panula, Carrageenan-induced changes in spinal nociception and its modulation by the brain stem, Neuroreport 9 (1998) 351–355.
[26] E. Pinteaux, L.C. Parker, N.J. Rothwell, G.N. Luheshi, Expression of interleukin-1 receptors and their role in interleukin-1 actions in murine microglial cells, J. Neurochem. 83 (2002) 754–763.
[27] J.J. Rady, J.M. Fujimoto, Confluence of antianalgesic action of diverse agents through brain interleukin-1β in mice, J. Pharmacol. Exp. Ther. 299 (2001) 659–665.
[28] T.A. Samad, K.A. Moore, A. Sapirstein, S. Billet, A. Allchorne, S. Poole, J.V. Bonventre, C.J. Woolf, Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity, Nature 410 (2001) 471–475.
[29] R. Sekido, K. Ishimaru, M. Sakita, Corticotropin-releasing factor and interleukin-1beta are involved in the electroacupuncture-induced analgesic effect on inflammatory pain elicited by carrageenan, Am. J. Chin. Med. 32 (2004) 269–279.
[30] Y.J. Seo, M.S. Kwon, E.J. Shim, S.H. Park, O.S. Choi, H.W. Suh, Changes in pain behavior induced by formalin, substance P, glutamate and pro-inflammatory cytokines in immobilization-induced stress mouse model, Brain Res. Bull. 71 (2006) 279–286.
[31] J.E. Sims, M.A. Gayle, J.L. Slack, M.R. Alderson, T.A. Bird, J.G. Giri, F. Colotta, F. Re, A. Mantovani, K. Shanebeck, K.H. Grabstein, S.K. Dower, Interleukin 1 signaling occurs exclusively via the type I receptor, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 6155–6159.
[32] Y.S. Son, H.J. Park, O.B. Kwon, S.C. Jung, H.C. Shin, S. Lim, Antipyretic effects of acupuncture on the lipopolysaccharide-induced fever and expression of interleukin-6 and interleukin-1 beta mRNAs in the hypothalamus of rats, Neurosci. Lett. 319 (2002) 45–48.
[33] A.J. Souter, M.G. Garry, D.L. Tanelian, Spinal interleukin-1beta reduces inflammatory pain, Pain 86 (2000) 63–68.
[34] R.V. Storkson, A. Kjorsvik, A. Tjolsen, K. Hole, Lumbar catheterization of the spinal subarachnoid space in the rat, J. Neurosci. Methods 65 (1996) 167–172.
[35] C.S. Sung, Z.H. Wen, W.K. Chang, S.T. Ho, S.K. Tsai, Y.C. Chang, C.S. Wong, Intrathecal interleukin-1beta administration induces thermal hyperalgesia by activating inducible nitric oxide synthase expression in the rat spinal cord, Brain Res. 1015 (2004) 145–153.
[36] T. Tadano, M. Namioka, O. Nakagawasai, K. Tan-No, K. Matsushima, Y. Endo, K. Kisara, Induction of nociceptive responses by intrathecal injection of interleukin-1 in mice, Life Sci. 65 (1999) 255–261.
[37] C.A. Winter, E.A. Risley, G.W. Nuss, Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs, Proc. Soc. Exp. Biol. Med. 111 (1962) 544–547.
[38] M. Zelenka, M. Schafers, C. Sommer, Intraneural injection of interleukin-1beta and tumor necrosis factor-alpha into rat sciatic nerve at physiological doses induces signs of neuropathic pain, Pain 116 (2005) 257–263.
[39] Y.Q. Zhang, G.C. Ji, G.C. Wu, Z.Q. Zhao, Excitatory amino acid receptor antagonists and electroacupuncture synergetically inhibit carrageenan-induced behavioral hyperalgesia and spinal fos expression in rats, Pain 99 (2002) 525–535.
[40] A. Zhang, C. Xu, S. Liang, Y. Gao, G. Li, J. Wei, F. Wan, S. Liu, J. Lin, Role of sodium ferulate in the nociceptive sensory facilitation of neuropathic pain injury mediated by P2X(3) receptor, Neurochem. Int. 30 (2008) (Epub ahead of print).
[41] M. Zimmermann, Ethical guidelines for investigation of experimental pain in conscious animals, Pain 16 (1983) 109–110.