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.

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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 years.
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 [29,36,18]. 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” (UB68, located near the ankle joint, between the tip of the external malleolus and tendon 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 of the rats according to the previous method [34] for ODN administration. 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′-CAGCTTATATCCCTCC-3′. The sense ODN to IL-1RI (5′-ATGGGATATGAAGACTG-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 [51,2], 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 centrifuged 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-IL-1β immunofluofinity-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, immunofluofinity-purified polyclonal sheep anti-IL-1β antibody (1:2000) with 13% 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 o-phenylene 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 H2O. 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 II 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, 100 units of SuperScript II reverse transcriptase. The sequences of the forward and reverse primers were as follows: IL-1RI forward, 5′-ACACATCCTATAGCTACG-3′; reverse, 5′-GAACAGCTCAGCCACCTT-3′ [1]; β-actin forward, 5′-CACCTGTCACCCGGCATG-3′; reverse, 5′-TAACCGAACCTATGCTAG-3′. The primers were synthesized and purified by Sangon Biotech Co. Ltd. (Shanghai, China). One microtitre 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 Pu 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 phenylmethylsulfonyl fluoride) at 4 °C. Following 12,000 rpm, 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 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.
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-quantitative analysis of RT-PCR showed 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. 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 quantitated and demonstrated. The mRNA levels of the different groups were expressed as a percentage to that of corresponding /H9252-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.

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.

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.
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 a 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 common types of pathological pain. In current pain modulation pain hypersensitivity, intrathecal injection of antisense ODN was used alone. When EA was combined with antisense ODN than when EA or combined with antisense ODN was used alone.

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 midbrain 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 ± 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±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±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 with 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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