Brain Research Bulletin 80 (2009) 89–94

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Electroacupuncture downregulates TLR2/4 and pro-inflammatory cytokine expression after surgical trauma stress without adrenal glands involvement

Jun Wang, Hui Zhao, Qi-Liang Mao-Ying, Xiao-Ding Cao, Yan-Qing Wang, Gen-Cheng Wu*

Department of Integrative Medicine and Neurobiology, Institute of Acupuncture Research WHO Collaborating Center for Traditional Medicine, Institutes of Brain Science, State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai 200032, China

ARTICLE INFO

Article history:
Received 20 February 2009
Received in revised form 16 April 2009
Accepted 16 April 2009
Available online 7 June 2009

Keywords:
Electroacupuncture
Surgical trauma stress
TLR
Pro-inflammatory cytokine
Adrenalectomy

ABSTRACT

Cumulative evidences suggest that electroacupuncture (EA) can modulate immune function, but the mechanism needs further study. In the present study, the contribution of EA on toll-like receptors 2 and 4 (TLR2/TLR4) and pro-inflammatory cytokine expression after surgical trauma stress were investigated. The mRNA level of both TLR2/4 and pro-inflammatory cytokine was measured by quantitative real-time PCR. ELISA and Western blot assay were chosen for TLR2/TLR4 protein expression and pro-inflammatory cytokine production, respectively. The results showed that surgical trauma stress increased TLR2 mRNA and TLR2/4 proteins in the spleen and augmented pro-inflammatory cytokines (e.g. IL-1β) mRNA and protein expression in the spleen and plasma. These effects could be deteriorated by adrenalectomy (ADX). EA at "Zusanli" acupoint significantly inhibited surgical trauma-induced TLR2 mRNA and TLR2/4 protein expression in spleen and pro-inflammatory cytokine expression in the spleen and plasma. ADX, however, could not block the effect of EA. These results suggested that surgical trauma stress primes the innate immune system for enhanced TLR2 expression and pro-inflammatory cytokine production. EA inhibits TLR2/4 and pro-inflammatory cytokines to produce an anti-inflammatory effect in a surgical trauma stress model, without adrenal gland involvement.

1. Introduction

Severe injury causes a dramatic host response that disrupts immune homeostasis and predisposes the injured host to opportunistic infections. In spite of the extensive studies in recent years and the growing understanding of different aspects of processes evoked by traumatic injury, the exact mechanism behind surgery-related immuno-dysfunction is not yet fully understood. Recent studies show that innate immune system may play an important role in the initiation of the process.

Toll-like receptors (TLRs) are evolutionarily conserved proteins that recognize microbial molecules, initiate the innate immune response, and modulate the adaptive immune system. Recent evidence has suggested that, in addition to their function as sensors of exogenous or foreign pathogen-associated molecular patterns (PAMPs), TLRs can recognize and mediate responses to endogenous stimuli [3,20,23,25]. Heat shock protein 60, a protein released by cells undergoing necrotic cell death, may activate innate immune cells through a TLR4-dependent mechanism [20]. Moreover, necrotic cells were recently shown to activate NF-κB and inflammatory gene induction in a TLR2-dependent manner [14]. As a whole, these observations have prompted us to consider whether injury triggers the endogenous TLR signaling to prime the immune system for enhanced TLR activity.

As the downstream effectors influenced by TLRs, pro-inflammatory cytokines are known to be elevated in a variety of settings in which stress response was elicited. Particularly, cytokines like interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α), and interleukin 6 (IL-6) were proposed to be proximal mediators in the early stages of inflammation. These cytokines are important mediators in the early stages of inflammation since they modulate many of the early responses that are induced by injury. Although the inflammatory response is a critical first line of defense against pathogens, when the inflammatory reaction is uncontrolled, it can cause more damage to the host than the initial stimulus. Adrenal gland, located on downstream of hypothalamus–pituitary–adrenal (HPA) axis, could release glucocorticoids upon stress stimulation and subsequently down-regulate pro-inflammatory cytokine production and secretion in immune cells [18]. Although many works have revealed that TLRs are related to inflammatory response, how the TLRs as well as downstream cytokines react and modulate under the surgical stress needs to be elucidated. To address the role of

* Corresponding author at: Department of Integrative Medicine and Neurobiology, Shanghai Medical College Fudan University, P.O. Box 291, 138 Yi-Xue-Yuan Road, Shanghai 200032, China. Tel.: +86 21 54237526; fax: +86 21 54237023.
E-mail addresses: jwangf@shmu.edu.cn (J. Wang), mayzhao@shmu.edu.cn (H. Zhao), yqlmao@shmu.edu.cn (Q.-L. Mao-Ying), xdcao@shmu.edu.cn (X.-D. Cao), wangyanqing@shmu.edu.cn (Y.-Q. Wang), gcwu@shmu.edu.cn (G.-C. Wu).

0361-9230/$ – see front matter © 2009 Published by Elsevier Inc.
doi:10.1016/j.brainresbull.2009.04.020

adrenal gland in the regulation of TLRs, the adrenalectomy (ADX) was adopted.

Acupuncture has traditionally been used in China and is being increasingly applied in Western countries to treat a variety of conditions [22]. It has been utilized more frequently in recent years to treat inflammatory disease [10]. From a basic science perspective it has been reported that EA significantly reduces complete Freund's adjuvant-induced hind paw edema and mouse air pouch inflammation [28,12]. EA could also improve immune dysfunction after surgical stress both in human and animals [27,29]. Therefore, it might be reasonably expected that the protective action of EA is exerted by the modulation of TLR2/4 expression and pro-inflammatory cytokine expression. In addition, the interplay between adrenal gland and EA would give insight in terms of mechanisms underlying the immunomodulation effect of EA.

In this study, we applied a rat surgical-trauma stress model to determine how injury alters TLR2 and TLR4 expression and pro-inflammatory cytokine response. We also evaluated the effect of EA on post-surgery immune response.

2. Materials and methods

2.1. Animals

Experiments were performed on adult male Sprague–Dawley rats (Experimental Animal Center, Shanghai Medical College of Fudan University, China) weighing 200–220 g. Rats were housed in temperature-controlled (22 ± 2 °C) and light-controlled (12:12 h light–dark cycle) room with free access to food and water. Prior to experimental manipulation, rats were allowed to acclimate to the housing facilities. All experimental protocols and animal handling procedures were approved by Animal Care and Use Committee (ACUC) of Fudan University, and were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Surgical trauma stress model

The surgical trauma stress was performed as previously described [27]. Briefly, the rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.). Animals were then incised longitudinally to a length of 6 cm along the dorsal median line and 5 cm along the abdominal median line. After surgery, the wounds were sutured and the animals were kept warm under standard housing conditions.

2.3. Experiment procedure

To determine the time course of pro-inflammatory and TLR2/4 mRNA expression after surgery, 24 rats were randomly divided into four groups, each group consist of 6 rats. The spleen samples were collected before surgery, and at 2, 24 and 48 h post-surgery. To observe the effect of EA on TLR and pro-inflammatory cytokine expression 24 h post-operation, the rats were randomly divided into four groups (n = 6–8 per group): (1) non-stressed group (Control); (2) surgical trauma stressed group (Trauma); (3) EA applied on surgical trauma stressed group (Trauma + EA); (4) EA applied on normal rats group (Control + EA). Immediately after decapitation, the trunk blood was collected into EDTA coated tubes and stored on ice. Plasma was immediately separated through refrigerated centrifugation, aliquoted and stored at −20 °C until the time of assay. The spleen was sectioned into two parts, one for protein assay, the other one for mRNA assay. The samples were frozen in liquid nitrogen and stored at −70 °C. To address the mechanism involved in modulation effect of EA, rats were divided into six groups (6–8 per group): (1) Control; (2) Trauma; (3) Control + ADX; (4) Trauma + ADX; (5) Trauma + Sham ADX; (6) Trauma +ADX + EA.

2.4. EA treatment

EA stimulation was applied immediately after the surgery and lasted for 30 min. According to our previous study [27], 'Zusanli' (ST 36) and 'Lanwei' (Extra 37) acupoints were selected. A pair of stainless steel needles of 0.3 mm diameter was inserted to a depth of 3 mm into the unilateral acupuncture points 'Zusanli' (located 5 mm below and lateral to the anterior tubercle of the tibia, between muscle anterior tibialis and muscle extensor digitorum longus, superficially, the lateral sural cutaneous nerve and the cutaneous branch of the saphenous nerve; deeper, the deep peroneal nerve) and 'Lanwei' (located about 3.3 mm below Zusanli, in muscle anterior tibialis and muscle extensor digitorum longus, nerves: the lateral cutaneous nerve of calf; deeper, the deep peroneal nerve). The acupuncture needles were bent into an 'L' shape to allow for consistent and reproducible insertion depth. After insertion, needles were held in place by plastic adhesive tape. The two needles were connected with the output terminals of an EA apparatus (Model G-6805-1, Shanghai Medical Electronic Apparatus Company, China). Alternating strings of dense–sparse frequencies (60 Hz for 1.05 s and 2 Hz for 2.85 s alternately) were selected. The intensity was adjusted to induce slight muscle contract of the hindlimb (≤1.5 mA).

2.5. Adrenalectomy

The bilateral adrenalectomies (ADX) were performed as described [16]. For bilateral ADX, incisions were made in the posterolateral abdominal wall and the procedures were carried out with the aid of a dissecting microscope. The adrenal glands were located by gross inspection, the artery to the adrenal gland was clamped and the glands were removed. ADX rats were provided with an additional water bottle of 0.9% sodium chloride. Sham ADX performed the same surgery without adrenal gland being removed. Animals were given 3 weeks to recover from this surgery.

2.6. Real-time PCR for mRNA quantification

TLR2/4 and pro-inflammatory cytokine mRNA were measured using real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis. Total RNA from the rat spleen was extracted with the Trizol reagent according to the manufacturer's recommendations (Invitrogen, USA). The quality of RNA was assessed by agarose gel electrophoresis. 1 μg of total RNA was reversely transcribed into cDNA for RT-PCR. The cDNA was stored at −20 °C until use. SYBR Green QRT-PCR detection (iCycler iQ® real-time PCR detection system, Bio-Rad, CA, USA) was used to quantify the relative abundance of target mRNAs in the samples. Oligonucleotide primers were chosen on the basis of rat nucleotide sequences in the GenBank database, and according to previously published sequences. The size and sequence of each primer and the number of cycles used are given in Table 1.

The relative amount of each mRNA was normalized to the housekeeping gene GAPDH mRNA. Each sample was run and analyzed in triplicate. The relative mRNA levels of gene expression were determined using the threshold cycle (C_T) and arithmetic formulas. The C_T from the GAPDH was subtracted from the C_T for each animal model (ΔC_T) and then the control group for each protein of interest was set to 1 for reference. The relative mRNA levels of IL-β, IL-6 and TNF-α were found by subtracting the control ΔC_T from each Animal ΔC_T. These values were entered into the equation $2^{-\Delta C_T}$ to solve for the relative exponential PCR amplification of each gene for each animal.

Table 1
Sequences of the forward and reverse primers and PCR conditions used for RT-PCR.

GenBank accession	Target gene	Primers	PCR conditions (temperature/time)			Predicted size (bp)
			Denature	Anneal	Extend	
NM017008	GAPDH	Forward: 5′-cccttcattgacctcaactac-3′ Reverse: 5′-cttctccatggtggtgaagac-3′	94 °C/45 s	60 °C/lmin	72 °C/1 min	217
NM198769	TLR2	Forward:5′-tggagactctggaagcaggt-3′ Reverse: 5′-cgcctaagagcaggatcaac-3′	94 °C/45 s	58 °C/lmin	72 °C/1 min	244
NM019178	TLR4	Forward: 5′-gccggaaagttattgtggtg-3′ Reverse: 5′-ccactcgaggtaggtgttt-3′	94 °C/45 s	58 °C/lmin	72 °C/1 min	203
NM031512	IL-1β	Forward: 5′-agagcttcaggaaggcag -3′ Reverse: 5′-tgttgttcatctcgaagcct -3′	94 °C/45 s	58 °C/lmin	72 °C/1 min	220
NM012589	IL-6	Forward: 5′-gacaaagccagagtccttca-3′ Reversed 5′-actaggtttgccgagtagac-3′	94 °C/45 s	58 °C/lmin	72 °C/1 min	229
X66539	TNF-α	Forward: 5′-cgagatgtggaactggcaga-3′ Reverse: 5′-ctacgggcttgtcactcga-3′	94 °C/45 s	58 °C/lmin	72 °C/1 min	256

2.7. Western blotting for TLR2 and TLR4 protein

Western blot analysis for TLR2 and TLR4 was performed according to the following protocol. Spleens were homogenized (100 mg/ml) at 4 °C in PBS containing 1 mM PMSF, 1 μg/ml pepstatin, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 0.5% Triton X-100, and 0.05% sodium azide. Homogenate from each recipient rat was centrifuged at 15,000 × *g* for 20 min twice, and the supernatant was used for Western blot analysis of TLR2 and TLR4 protein. The concentration of protein was measured using the bicinchoninic acid (BCA) method. Aliquots of supernatant containing 50 μg of protein were resolved in 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE; 10%). The separated proteins were then transferred (300 mA for 1.5 h) onto polyvinylidene difluoride (PVDF) membrane. After incubated in Blocking reagent (1× PBS, 5% BSA, 0.05% Tween-20) at room temperature for 60 min to block unspecific binding, the membrane was incubated in Blocking reagent containing a goat polyclonal anti-TLR antibody (Santa Cruz Biotechnology, Santa Cruz, CA) in 1:200 dilutions at 4 °C overnight. After washing in PBS containing 0.05% Tween-20 (PBST) buffer, the membrane was incubated for 60 min at room temperature in PBST buffer containing a 1:500 dilution of HRP-conjugated rabbit anti-goat IgG (Santa Cruz Biotechnology, Santa Cruz, CA). To visualize the result, chemiluminescence reaction using the ECL system was adopted (Pierce).

2.8. Cytokine ELISA

IL-1β, IL-6 and TNF-α protein concentration were measured by using ELISA kits according to the manufacturer's instructions (RapidBio Lab, California, USA). Briefly, standards and samples were added (100 μl/well) into pre-coated 96-well microtiter ELISA plates and incubated for 30 min at RT. The plates were washed five times, 100 μl of biotinlylated detection Ab was added per well, and the plates were incubated for 30 min at RT; then the plates were again washed thoroughly, avidin-HRP was added, and the plates were incubated at RT for 15 min. After further washing, developer substrate TMB was added; following color development for 5–15 min, the reaction was stopped with stop solution, and the absorbance was determined with an ELISA plate reader at 450 nm (Bio-Tek Instrument).

2.9. Statistical analysis

Data were presented as mean ± SEM. The statistical difference between groups was assessed by one-way ANOVA, followed by Student–Newman–Keul test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Surgical trauma stress augments TLR2 and inflammatory cytokine mRNA expression in spleen

To determine how the surgical trauma affects the transcription of TLR2/4 and pro-inflammatory cytokines in spleen, we detected mRNA expression with QRT-PCR. Since host immune response to injury is time-dependent, we performed experiments at immediate (2 h), acute (24 h), and sub-acute (48 h) time points. As shown in Fig. 1, IL-1β, IL-6 and TNF-α mRNA in spleen increased immediately 2 h after trauma, IL-1β and TNF-α reached peak at 24 h and came back to normal 48 h later. TLR2 mRNA expression displayed the same pattern as IL-1β, it reached peak at 24 h after surgical trauma. TLR4 mRNA did not change significantly at each time point. Based on this observation, the time point of 24 h post-surgery was used in the following analysis.

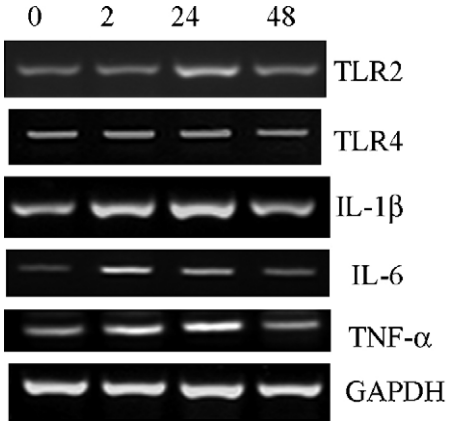

Fig. 1. Time course of spleen TLR2/4 and pro-inflammatory cytokine production after surgical trauma stress. Rats underwent surgical trauma stress and were sacrificed 2, 24, or 48 h later for the measurement of mRNA expression by RT-PCR. Each time point consists of 6 rats. The result shown is representative of three independent experiments.

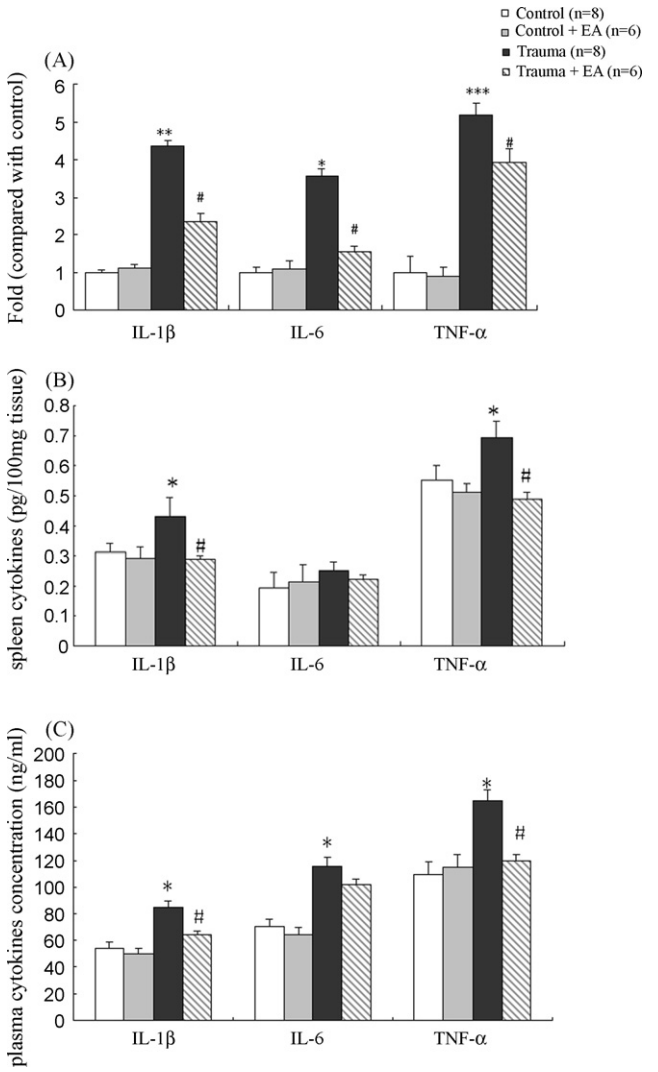

Fig. 2. EA suppresses pro-inflammatory cytokine expression both in spleen and in plasma. (A) Pro-inflammatory cytokine mRNA was measured 24 h after surgical trauma and EA treatment; (B) tissue pro-inflammatory cytokine concentration was measured using ELISA after surgical trauma and EA treatment; and (C) plasma pro-inflammatory cytokine concentration after surgical trauma and EA treatment. All mRNA levels were measured by real-time PCR and are expressed as a ratio relative to the loading control. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with Control; $^{\#}P < 0.05$, compared with Trauma.

3.2. EA suppresses pro-inflammatory cytokine expression both in spleen and in plasma

Upon TLRs stimulation, they can activate NF-κB, which in turn triggers the gene expressions of many pro-inflammatory cytokines. Therefore, we measured the IL-1β, IL-6 and TNF-α mRNA expression in spleen 24 h after trauma by using QRT-PCR. The protein level of cytokines both in spleen and in plasma was measured by ELISA. As shown in Fig. 2, surgical trauma not only augmented IL-1β (4.36-fold of control, $P < 0.01$), IL-6 (3.57-fold of control, $P < 0.05$), and TNF-α (5.18-fold of control, $P < 0.001$) mRNA expression in spleen (Fig. 2A), but also increased IL-1β and TNF-α protein pro-

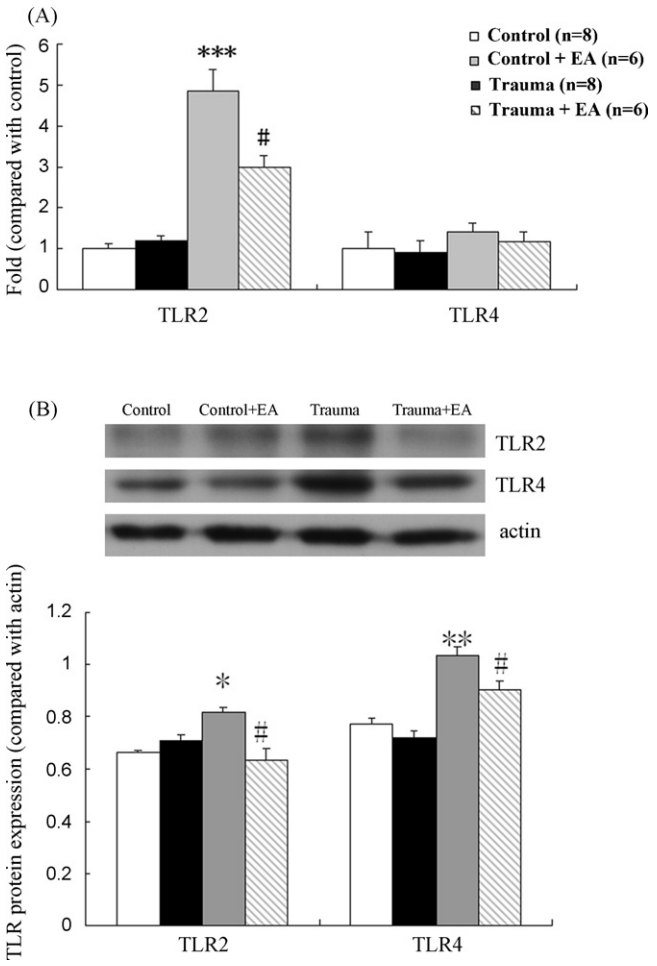

Fig. 3. EA decreases spleen TLR2 expression after surgical trauma stress. (A) TLR2/4 mRNA expression in spleen; (B) TLR2/4 protein expression were measured by Western blot. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with Control; $^{\#}P < 0.05$, compared with Trauma.

duction in spleen (Fig. 2B) and plasma (Fig. 2C). EA down-regulated inflammatory cytokine mRNA expression to certain extent, it significantly inhibited IL-1β, IL-6 and TNF-α mRNA expression in spleen (Fig. 2A). EA could inhibit IL-1β and TNF-α protein production and secretion but had no effect on IL-6 secretion (Fig. 2B and C).

3.3. EA inhibits TLR expression in spleen of surgical trauma stressed rats

Two ways can detect TLR activation, one is up-regulated TLR expression and the other is up-regulated TLR function. In the present study, we examined the TLR2/4 expression in spleen. As detected by Western blot analysis, the expression of TLR2/4 protein in spleen was significantly enhanced 24 h after stress (Fig. 4B). Surgical trauma significantly increased TLR2 mRNA and protein expression 24 h after stress, as well as enhanced TLR4 protein expression (Fig. 3A). EA treatment could reverse the effect exerted by surgery (Fig. 3).

3.4. The regulatory effects of EA is independent of ADX

Since the HPA is thought to be involved in the regulation of pro-inflammatory cytokine secretion, ADX was adopted here to determine whether the adrenal glands were responsible for the previously observed trauma-induced inflammatory response. The result showed that ADX alone did not affect either systemic or tissue pro-inflammatory cytokine expression in normal rats. But it could significantly increase IL-1β mRNA and protein expression both in tissue and in circulation in surgical rats (Fig. 4A–C), while IL-6 and TNF-α expression were not influenced by ADX (Fig. 4A–C). ADX could also increase TLR2 expression in surgical rats (Fig. 4D). It is suggested that adrenal gland may play a role in post-surgical inflammatory response. In ADX group, EA stimulation still produced a strong suppressive effect on IL-1β and TNF-α production in spleen and circulation. The result showed that adrenal gland did not play any role in the anti-inflammatory effect of EA.

4. Discussion

In the present study, the main finding is that surgical trauma causes an augmented pro-inflammatory response, as well as the increased TLR2/4 expression after surgical operation. The effect is potentiated by adrenalectomy.

It has been suggested that injury triggers a cascade of pro-inflammatory reactions. Aberrant reactions can lead to a systemic inflammatory response syndrome, with a potentially lethal outcome. Besides that, injury induces a “cytokine storm”, which then sets in motion the downstream, phenotypic changes in immunity [19]. A number of studies have documented that various types of experimentally induced tissue injury do cause an increase in circulating inflammatory cytokines [21]. The cytokines, particularly IL-1β, IL-6, and TNF-α, are shown to be elevated in the circulation of patients at early time points (within 1 day after major injury or surgery) [4,24]. Similar patterns of cytokine induction have been observed in several different animal injury models [1,6]. In the present study, significantly elevated pro-inflammatory cytokines productions were observed both in circulation and in spleen during the post-operation period. IL-1β, IL-6 and TNF-α mRNA in spleen increased immediately, reached peak at 24 h, then came back to normal 48 h later. Therefore, these results provided further evidence that pro-inflammatory cytokines may constitute the important step in the cascades of events induced by surgical trauma stress.

TLRs are one of the possible candidates who are responsible for initiating the inflammatory response. In the present study, we also demonstrated that TLR2/4 expression was up-regulated by surgical trauma. It is well known that TLRs have the capacity to recognize PAMPs displayed by a variety of microorganisms, their regulation is fundamental to the activation of innate responses [9]. According to the recently proposed “danger model” [15], the innate immune system is capable of sensing danger signals, such as endogenous ligands released from damaged or stressed cells within the organism, which leads to the initiation of a host immune response. As mentioned above, TLR2 or TLR4 could function as a danger signal receptor in response to the cellular damage mediated by operation. Thus our data were consistent with other reports. Also, the results gave us the hint that the surgical trauma stress may trigger some danger signal release, which activates TLR2/4 and finally lead to increased inflammatory response. The results of the expression of TLR2 and TLR4 after operation is inconsistent: Ikushima et al. showed that TLR2 and TLR4 are down-regulated after operation [8], while Dybdahl et al. observed an initial down-regulation of monocyte CD14, TLR-2, and TLR-4 after the coronary artery bypass grafting operation. Subsequently, CD14 normalized, whereas TLR-2 and TLR-4 were unregulated [5]. The difference between the TLR expression profiles may be due to the severity of injury and type of cell examined. However, we did not find significant changes of TLR4 mRNA expression after surgical trauma stress. The increase of TLR4 protein expression may be derived from complicated post gene transcript modulation process.

Immunomodulation effect of EA has been addressed for several decades. For example, low-frequency EA suppressed carrageenan-

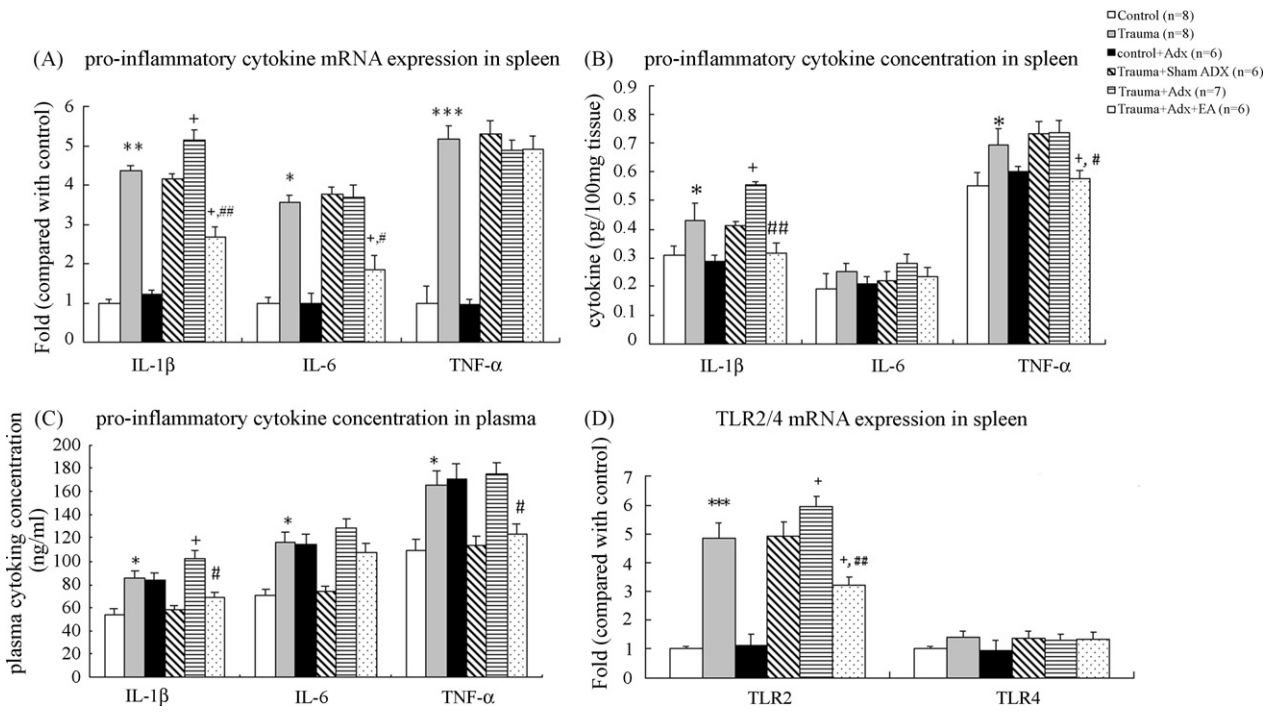

Fig. 4. Effect of ADX on TLR2/4 and pro-inflammatory cytokine expression. (A) and (B) Pro-inflammatory cytokine mRNA and protein production in spleen; (C) pro-inflammatory cytokine concentration in plasma; (D) TLR2/4 mRNA expression in spleen. All mRNA levels were measured by real-time PCR and are expressed as a ratio relative to the loading control. The cytokine protein level was measured by ELISA assay. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, compared with Control; $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with Trauma + ADX; $^{+}P<0.05$, compared with Trauma.

induced paw inflammation as well as zymosan-induced peripheral inflammation in mice [11,13]. The anti-inflammatory effect of EA is also observed both in clinic and in animal models [30]. In the present study, the protective role of EA on inflammatory response mediated by surgical trauma stress was observed. EA not only reversed TLR2/4 expression, but also inhibited pro-inflammatory cytokine production and secretion. This result promoted us to further explore the mechanisms behind.

It is well established that HPA axis is involved in the regulation of systemic inflammatory response. Enhanced adrenal glucocorticoid release during stress could prevent inflammatory cytokines production. Also, glucocorticoids receptor could inhibit TLR4-mediated inflammatory cytokine secretion [2]. Hermoso et al. found that glucocorticoids synergistically enhance the IL-1β-induced TLR2 expression in the cultured A549 cells [7]. Our study demonstrated that ADX rats displayed potentiated effect to surgical trauma, namely, further increase in IL-1β and TLR2 expression. In addition, HPA axis was proposed to involve in the conveying EA signals. However, ADX could not block protective effect of EA, suggesting that HPA axis may not be required for the early modulation of EA in the current scenery. Our result is inconsistent with Hermoso, the possible explanations are that they carried out the experiment in vitro, and the cell line they were using was human alveolar basal epithelial cell.

The anti-inflammatory effect of EA was not observed on non-stressed control rats, which means that the efficiency of EA is specifically limited on surgical trauma-stressed rats. Some published paper set up sham EA control by inserting needles into the acupoint without electric stimulation. The sham EA control is controversial. In ancient China, there is no such kind of electric machine existed, the manual acupuncture was the only way used to achieve efficacy. We did not carry out this control because we thought that manual acupuncture may also have some effect. Further study is needed to evaluate the difference between manual acupuncture and electroacupuncture as well as acupoint specificity on anti-inflammatory effect of EA.

In the present experiment, the anti-inflammatory effect of EA was not blocked by ADX, suggesting that other pathway is involved in regulatory function. Based upon previous parametric studies of Meltzer, who found that the sympathetic nervous system (SNS), not the HPA axis, was primarily responsible for the immunosuppressive effect of stress [17], it is assumed that SNS may mediate the immunomodulation effect of EA. The SNS has significant anatomical and functional interaction with cells of the immune system and plays an important role in control of magnitude of early inflammatory response to injury by ensuring expression of adequate cytokine balance. Recent evidence showed that low-frequency EA suppressed inflammation via sympathetic post-ganglionic neurons, while high-frequency EA suppression was mediated by the sympathoadrenal medullary axis [13]. Our previous work demonstrated that sympathetic nervous system mediated surgical trauma induced lymphocyte apoptosis [26]. Further study is needed to explore the contribution of sympathetic nervous system in the anti-inflammatory effect of EA.

5. Conclusion

Surgery-induced immune alterations are complicated phenomenon likely due to the interaction between the varieties of endogenous processes. Although the mechanisms involved in the effects of surgery on immune status are complex, the present study demonstrates that surgical trauma stress primes the innate immune system by enhanced TLR2 expression and systemic inflammatory cytokine secretion. EA produces an anti-inflammatory effect probably through inhibiting TLR2 and TLR4 expression. Adrenal gland is not involved in the modulatory effect of EA. The present experiment provides pertinent information which may

ultimately contribute to a better understanding of surgery-induced immune alterations

Acknowledgement

The study is supported by National Key Basic Research program of China (2005CB523306, 2007CB512502).

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