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Basic Study

Effect of electroacupuncture on NF-κB and NLRP3 inflammasome in uterine tissues of rats with primary dysmenorrhea

电针对原发性痛经大鼠子宫组织NF-κB和NLRP3炎症小体的影响

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Abstract

Objective: To observe the effect of electroacupuncture (EA) on nuclear factor kappa B (NF-κB) and nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome in uterine tissues of rats with primary dysmenorrhea (PD), thus to explore the possible mechanism of EA for PD.

Methods: Fifty female Sprague-Dawley (SD) rats were randomly divided into a normal group, a model group, an EA at non-acupoint group, an EA at acupoint group and a Western medicine group, with 10 rats in each group. Except for the normal group, rats in the other four groups were treated with estradiol benzoate combined with oxytocin for 11 d to establish PD rat models. From day 1 of the modeling, rats in the normal group and the model group were only properly grasped without any intervention; Guanyuan (CV 4) and Sanyinjiao (SP 6) were selected for EA treatment in the EA at acupoint group; rats in the EA at non-acupoint group were treated with EA at 5 mm away from the acupoints selected above; rats in the Western medicine group were treated with ibuprofen via gavage. Rats in each group were treated for 10-day successively. On the 11th day, except for the normal group, rats in the other groups were intraperitoneally injected with oxytocin (2 U/rat), and the writhing number within 30 min in each group was compared; the pathological changes in rat uteruses were observed by hematoxylin-eosin (HE) staining, and the pathological damage scores were evaluated. Protein expression levels of NF-κB p65, phospho-NF-κB p65, NLRP3, cysteine aspastic acid-specific protease 1 (caspase-1), interleukin (IL)-1β and IL-18 were detected by Western blot.

Results: Compared with the normal group, the writhing number increased significantly (P<0.05), and the extensive exfoliation of the endometrium, severe edema, and histopathological score all increased significantly in the model group (P<0.05) as well as the protein levels of NLRP3, caspase-1, IL-1β and IL-18, and the ratio of phospho-NF-κB p65/NF-κB p65 in rat uterine tissues (all P<0.05); compared with the model group, the numbers of writhing reaction decreased within 30 min (P<0.05), the endometrial exfoliation was rare, the edema degree was mild, and the histopathological scores decreased significantly (all P<0.05) in the EA at acupoint group and the Western medicine group; compared with the model group, the phospho-NF-κB p65/NF-κB p65 ratio and the NLRP3, caspase-1, IL-1β and IL-18 protein levels of rat uterine tissues in the EA at acupoint group were significantly lower (P<0.05); compared with the model group, the caspase-1, IL-1β and IL-18 protein levels of the rat uterine tissues decreased significantly (all P<0.05), and the differences in the NLRP3 and phospho-NF-κB p65/NF-κB p65 levels were statistically insignificant (all P>0.05) in the Western medicine group; compared with the Western medicine group, the phospho-NF-κB p65/NF-κB p65 ratio, also the NLRP3, IL-1β and IL-18 protein levels of the uterine tissues decreased significantly in the EA at acupoint group (all P<0.05), while the difference in the caspase-1 level was statistically insignificant (P>0.05); there were no significant differences between the EA at non-acupoint group and the model group in any indicators (all P>0.05).

Conclusion: EA at acupoints significantly improves the pain and pathological damages of PD rats. The mechanism may be related to the reduced uterine inflammation via inhibiting NF-κB phosphorylation and NLRP3 activation in uteruses of PD rats.

Keywords: Acupuncture Therapy; Electroacupuncture; Dysmenorrhea; NF-kappa B; Interleukins; NLRP3; Caspase-1; Rats

【摘要】目的：观察电针对原发性痛经(PD)大鼠子宫组织核转录因子 κB(NF-κB)和核苷酸结合寡聚化结构域样受体蛋白 3(NLRP3)炎症小体的影响，探讨电针治疗 PD 的可能机制。**方法：**将 50 只 Sprague-Dawley(SD)雌性大鼠随机

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分为正常组、模型组、电针非穴位组、电针穴位组和西药组，每组 10 只。除正常组以外，其余四组采用苯甲酸雌二醇联合缩宫素建立 PD 大鼠模型，造模持续 11 d。自造模第 1 天起，正常组和模型组只适度抓取，不作任何干预；电针穴位组取大鼠关元和三阴交行电针治疗；电针非穴位组选取上述两穴旁开 5 mm 非经非穴位处行电针治疗；西药组予布洛芬灌胃治疗。各组均连续治疗 10 d。于第 11 天，除正常组以外，其余各组大鼠腹腔注射缩宫素(2 U/只)后比较各组大鼠 30 min 内扭体次数；苏木精-伊红(HE)染色法观察大鼠子宫病理形态学的变化，并进行病理损伤评分；Western blot 法检测子宫组织核转录因子 κB p65(NF-κB p65)、磷酸化 NF-κB(phospho-NF-κB p65)、NLRP3、半胱氨酸天冬氨酸特异性蛋白酶 1(caspase-1)、白细胞介素(IL)-1β 和 IL-18 的蛋白表达水平。**结果**：与正常组相比，模型组扭体次数明显增多(P＜0.05)、子宫内膜大范围剥脱及较严重的水肿、组织病理评分明显上升(P＜0.05)，大鼠子宫组织中 NLRP3、caspase-1、IL-1β 和 IL-18 蛋白水平及 phospho-NF-κB p65/NF-κB p65 水平明显升高(均 P＜0.05)；与模型组相比，电针穴位组和西药组 30 min 内扭体反应次数均减少(均 P＜0.05)，子宫内膜剥脱少见且水肿程度较轻，组织病理评分显著降低(均 P＜0.05)；与模型组相比，电针穴位组大鼠子宫组织 pospho-NF-κB p65/NF-κB p65 水平及 NLRP3、caspase-1、IL-1β 和 IL-18 蛋白水平明显降低(均 P＜0.05)；与模型组相比，西药组大鼠子宫组织 caspase-1、IL-1β 和 IL-18 蛋白水平明显降低(均 P＜0.05)，NLRP3 和 phospho-NF-κB p65/NF-κB p65 水平差异无统计学意义(均 P＞0.05)；与西药组相比，电针穴位组大鼠子宫组织 phospho-NF-κB p65/NF-κB p65 水平及 NLRP3、IL-1β 和 IL-18 蛋白水平明显降低(均 P＜0.05)，caspase-1 水平差异无统计学意义(P＞0.05)；电针非穴位组与模型组相比，各项指标差异无统计学意义(均 P＞0.05)。**结论**：电针穴位可明显改善 PD 大鼠疼痛症状和病理损伤情况，其作用机制可能与抑制 PD 大鼠子宫组织中 NF-κB 磷酸化和 NLRP3 活化，减轻子宫炎症有关。

【关键词】 针刺疗法；电针；痛经；核转录因子；白细胞介素；核苷酸结合寡聚化结构域样受体蛋白 3；半胱氨酰天冬氨酸特异性蛋白酶 1；大鼠

【中图分类号】R2-03　　**【文献标志码】**A

Primary dysmenorrhea (PD) is a common gynecological disease without organic disorder in pelvic organs and with main clinical symptoms of lower abdominal cramps accompanied by nausea, headache and diarrhea[1]. In recent years, studies have shown that inflammatory response plays an important role in the development of PD[2]. The inflammatory response in PD is mainly mediated by pro-inflammatory factors including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6[3-4]. Nuclear factor kappa B (NF-κB) and nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome are key proteins that regulate the secretion of pro-inflammatory factors. NF-κB is a key transcription factor that regulates inflammatory signaling in cells. It usually binds to inhibitors of NF-κB (IκB) and exists in the cytoplasm as an inactive form. When the body is stimulated, IκB is phosphorylated and dissociated from NF-κB, and NF-κB is further activated by phosphorylation and translocated into the nucleus, regulating the expression and secretion of pro-inflammatory factors such as IL-1β and TNF-α[5]. IL-1β acts as a downstream factor of NLRP3 inflammasome. Activated NLRP3 inflammasome can render inactive pro-cysteine aspartic acid 1 (pro-caspase-1) to be self-sheared into the active cysteinyl aspartate-specific protease 1 (caspase-1) when various signals induce NLRP3 activation. Caspase-1 cleaves interleukin-1β precursor (pro-IL-1β) and interleukin 18 precursor (pro-IL-18) into active IL-1β and IL-18, thereby aggravating the downstream inflammatory response[6-8]. It is suggested that NF-κB and NLRP3 inflammasome may be the key link between inflammation and PD. Previous studies have found that electroacupuncture (EA) can reduce the inflammatory response by inhibiting the secretion of inflammatory factor IL-1β in the uterus, and alleviate the pain caused by uterine ischemia[9], but the mechanism of EA intervention of PD is not yet clear. In this study, we established a PD rat model to observe the effects of EA on NF-κB p65 and NLRP3 inflammasome in rat uterus, and explored the mechanism of EA treatment of PD by targeting NF-κB and NLRP3 inflammasome.

1 Materials and Methods

1.1 Laboratory animals and grouping

Fifty healthy adult female SPF Sprague-Dawley (SD) rats, 2-3 months old, weighing 220-250 g, were provided by the Animal Experimental Center of Hunan University of Chinese Medicine [animal certificate number: SYXK (Xiang) 2013-0004]. Rats received adapted feeding in the animal house for 1 week before the experiment with free access to water and diet. The temperature of the breeding room was 20-25 ℃ with natural day and night rhythm. The rats were then randomly divided into a normal group, a model group, an EA at non-acupoint group, an EA at acupoint group and a Western medicine group, with 10 rats in each group. The animal treatment during the experiment was in line with the *Instructive Notions with Respect to Caring for Laboratory Animals* promulgated by the Ministry of Science and Technology of China in 2006.

1.2 Main instruments and reagents

TGL20M desktop high speed refrigerated centrifuge (Changsha Xiangzhi Centrifuge Instrument Co., Ltd., China); electrophoresis apparatus, transfer box and gel imaging systems (BIO-RAD, USA); Hwato Brand SDZ-V

type EA instrument (Suzhou Medical Products Factory Co., Ltd., China).

Ibuprofen [production batch number: State Food and Drug Administration (SFDA) approval No. H10900089, specification: 300 mg/tablet, Tianjin Shike Pharmaceutical Co., Ltd., China]; estradiol benzoate (SFDA approval No. H12020529, specification: 2 mg/mL, Guangzhou Baiyunshan Mingxing Pharmaceutical Co., Ltd., China); oxytocin (production batch number: A605015, specification: 100 mg, Sangon Biotech Co., Ltd., China); RIPA lysate (production lot number: P0013B, Beyotime Biotechnology, China); protease inhibitor cocktail (without EDTA, 100×DMSO stock solution) (production lot number: B14002, Bimake, USA); phosphatase inhibitor cocktail (A, B tube, 100×) (production batch number: B15002, Bimake, USA); BCA protein quantification kit (production lot number: A53225, Thermo, USA); PVDF membrane (specification: 26.5 cm×3.75 m, 0.22 μm, Beyotime Biotechnology, China); NF-κB p65 antibody (production lot number: #4746, Cell Signaling Technology, USA); phospho-NF-κB p65 antibody (production lot number: #3033, Cell Signaling Technology, USA); IL-1β antibody (production lot number: ab9722, Abcam, UK); IL-18 antibody (production batch number: ab191860, Abcam, UK); NLRP3 antibody (production lot number: NBP2-12446, Novus Biologicals, USA); caspase-1 antibody (production lot number: NBP1-45433, Novus Biologicals, USA); beta-actin mouse monoclonal antibody (production batch number: A5316, Sigma, USA); ultra-sensitive ECL chemiluminescence kit (production batch number: P0018FFT, Beyotime Biotechnology, China); goat anti-rabbit secondary antibody (production batch number: AP132P, Merck Millipore, Germany); goat anti-mouse secondary antibody (production batch number: AP124P, Merck Millipore, Germany); hematoxylin-eosin (HE) staining kit (production batch number: C0105, Beyotime Biotechnology, China).

Disposable sterile acupuncture needle (specification: 0.30 mm in diameter, 13 mm in length, Maanshan Bond Medical Instruments Co., Ltd., China).

1.3 Modeling method

Except for the normal group, rats in the other four groups were prepared to be PD models using estradiol benzoate combined with oxytocin according to the literatures[10-11]. The rats were subcutaneously injected with estradiol benzoate at 0.5 mg/rat on day 1 (6 p.m.), 0.2 mg/rat during day 2-9, and 0.5 mg/rat on day 10, around the thigh; intraperitoneally injected with oxytocin (saline based, 500 mg/L, i.e. 5 U/mL) at 2 U/rat on day 11. Rats in the normal group were subcutaneously injected with normal saline at the same time point for 10 d around the thigh, once a day, and the same amount of saline was intraperitoneally injected on the 11th day. The successful standard for PD rat model was the writhing response after oxytocin injection[12].

1.4 Intervention methods

1.4.1 Normal group and model group

Rats in the normal group and the model group were only properly grasped during the treatment period without any treatment.

1.4.2 EA at acupoint group

Acupoints: Sanyinjiao (SP 6) and Guanyuan (CV 4).

Method: Rats were fixed in a self-made cloth set. According to the acupuncture point positioning of rats in *Experimental Acupuncture and Moxibustion*[12], Sanyinjiao (SP 6) was located at 10 mm above the medial malleolus apex of the hind limb and Guanyuan (CV 4) was located at 25 mm below the umbilicus. The umbilicus is at the lower 1⁄4 and the upper 3⁄4 intersection between the sternoclavicular joint and the pubic symphysis. After routine disinfection of each acupoint, acupuncture was performed with disposable sterile acupuncture needles of 0.30 mm in diameter and 13 mm in length. Sanyinjiao (SP 6) was perpendicularly punctured for 5 mm and Guanyuan (CV 4) was perpendicularly punctured for 2 mm. The positive electrode of the conducting wire was connected to the needle and the negative electrode of the conducting wire was clamped on the skin next to the acupoint. A continuous wave was selected at 50 Hz. The stimulation intensity was tolerable and slight twitch of the local muscles in rats.

Course of treatment: The treatment started immediately on the first day of modeling, once a day for 20 min and continuous 10 d. The acupuncture treatment was performed by the same person.

1.4.3 EA at non-acupoint group

Needles are inserted about 5 mm away from Sanyinjiao (SP 6) and Guanyuan (CV 4) to avoid the adjacent acupoints. The methods and the course of EA treatment were the same as those in the EA at acupoint group.

1.4.4 Western medicine group

Rats after grasping and fixation were subjected to ibuprofen by conventional gavage method (mixed with normal saline to a concentration of 1.25 g/L, 0.8 mL/day, once daily). The course of treatment was the same as that in the EA at acupoint group.

1. 5 Observation items and detection methods

1.5.1 Determining the number of writhing[10]

After the 11th day of injection, the numbers of writhing reactions within 30 min in each group are observed. The standard of writhing reaction was that the rat's abdomen caved in, the trunk and hind limbs were stretched, and one side limbs internally rotated.

1.5.2 Observing pathological changes of uterine tissues by HE staining

The rats were anesthetized by intraperitoneal

injection with 10% chloral hydrate at a dose of 3.5 mL/(kg·bw). The rat's uterine tissue of each group was quickly taken out on an ice tray. A portion of the same side rat uterine tissue in each group was dissected and washed with pre-cooled saline for 1-2 times, and fixed in 4% paraformaldehyde after blotted dry with filter paper. The tissues were embedded in paraffin and sectioned (5 μm thick). The sections were fixed on polylysine-treated slides for routine HE staining. After the slides were mounted, the pathological changes of the uterus in each group were observed under the microscope, and the pathological damages of the tissues were scored. The pathological changes were evaluated according to the pathological changes of endometrial degeneration and necrosis, lamina propria edema, increased lamina propria gland, inflammatory cell infiltration lamina propria and myometrial inflammation. The normal was 0 point. The damage degree from mild to severe was 0.5 point, 1 point, 2 points, 3 points and 4 points. Comprehensive statistics were performed after the evaluation[13].

1.5.3 Western blot analysis of IL-1β, IL-18, NLRP3, caspase-1, phospho-NF-κB p65 and NF-κB p65 protein expressions in uterus

Five rats were randomly selected from each group. Same side uterine tissue (100 mg) of each rat was accurately weighed, 1 mL of lysate containing 10 μL of protease and phosphatase inhibitor was added and the protein was cleaved on ice for 10 min. The supernatant containing the total tissue proteins was collected after the homogenate was centrifuged at 4 ℃, 12 000 r/min for 10 min, then the sample buffer was added and heated in boiling water bath for 10 min to fully denature the proteins. Protein quantification was performed according to the instructions of BCA protein quantitation kit. The protein concentration was calculated and trimmed based on the protein standard curve. After electrophoresis, membrane transfer, and blocking, membrane with primary antibodies of NLRP3 (1:1 000), caspase-1 (1:500), IL-1β (1:1 000), IL-18 (1:1 000), phospho-NF-κB p65 (1:1 000), NF-κB p65 (1:1 000) or β-actin (1:5 000) was incubated overnight at 4 ℃. After the membrane was washed with TBST buffer, the diluted secondary antibody (1:10 000) was incubated with the membrane on a shaker at room temperature for 60 min. After ECL color and exposure, the protein bands were analyzed using Quantity One gray analysis software. β-actin was used as an internal reference protein. The ratio between IL-1β, IL-18, LRP3 or caspase-1 and β-actin indicated the relative protein level. The phosphorylation level of NF-κB p65 was expressed as the ratio of phospho-NF-κB p65/NF-κB p65.

1.6 Statistical methods

Statistical processing was performed using the SPSS version 17.0 software. The obtained measurement data in normal distribution were expressed as mean ± standard deviation ($\bar{x} \pm s$). One-way ANOVA was used for multiple-group comparison; the least significant difference (LSD) *t*-test was used for comparing paired data with homogeneity of variances; the Dunnett's *t*-test was used when the variance was heterogeneity. The rank sum test was used if the normal distribution was not met. The difference was statistically significant at $P<0.05$.

2 Results

2.1 Comparison of writhing times of rats among groups

After the final injection, there was no writhing reaction in the normal group within 30 min; the writhing number in the model group was significantly different from that in the normal group ($P<0.05$), indicating successful modeling; compared with the model group, the writhing numbers in the EA at acupoint group and the Western medicine group were decreased, the differences were statistically significant (both $P<0.05$). There was no significant difference in the writhing number between the Western medicine group and the EA at acupoint group ($P>0.05$); compared with the normal group, the writhing number of the EA at non-acupoint group increased significantly ($P<0.05$), and there was no significant difference between the EA at non-acupoint group and the model group ($P>0.05$), (Table 1).

Table 1. Comparison of writhing times of rats among groups ($\bar{x} \pm s$, time)

Group	*n*	Writhing time
Normal	10	0
Model	10	38.83±4.66[1)]
EA at non-acupoint	10	40.50±4.19[1)]
EA at acupoint	10	25.66±2.68[2)]
Western medicine	10	26.57±3.81[2)]

Note: Compared with the normal group, 1) $P<0.05$; compared with the model group, 2) $P<0.05$

2.2 Comparison of pathological findings in rat uterine tissues

In the normal group, the endometrium was intact, and there was no obvious pathological change such as edema. The model group and the EA at non-acupoint group showed a wide range of endometrial exfoliation and more severe edema. In the EA at acupoint group and the Western medicine group, endometrial exfoliation was rare and the edema degree was mild (Figure 1).

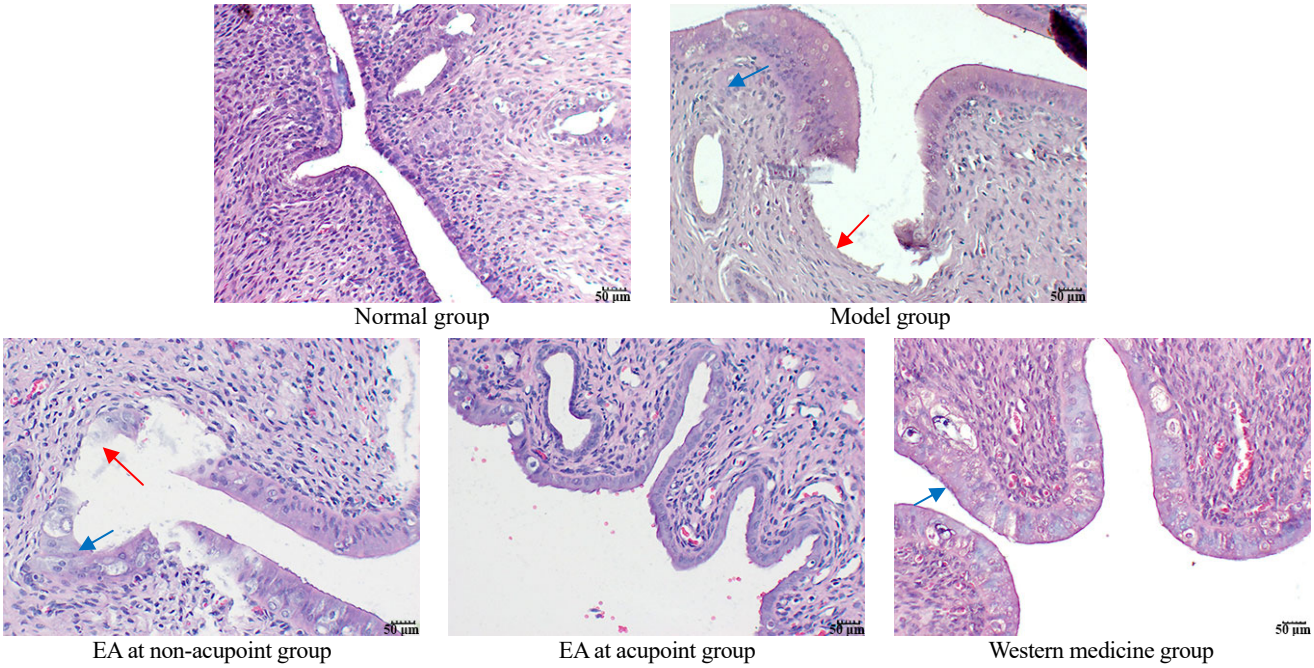

Figure 1. Pathologic pictures of rat uterus in each group (HE, ×200)

Note: The blue arrow shows endometrial edema, and the red arrow shows endometrial shedding

Compared with the normal group, the pathological score of the model group increased significantly (P<0.05); the pathological scores of EA at acupoint group and Western medicine group were significantly lower than the score of the model group (both P<0.05), and there were no statistical differences versus the normal group (both P>0.05). There was no significant difference in the pathological score between EA at acupoint group and Western medicine group (P>0.05). The pathological score of EA at non-acupoint group was significantly higher than that in the normal group (P<0.05), and there was no significant difference versus the model group (P>0.05), (Table 2).

Table 2. Comparison of pathological score of uterine tissues among groups ($\bar{x}$ ±s, point)

Group	n	Pathological score
Normal	10	0.25±0.25
Model	10	6.62±1.67[1)]
EA at non-acupoint	10	6.21±1.60[1)]
EA at acupoint	10	1.85±0.51[2)]
Western medicine	10	1.75±0.78[2)]

Note: Compared with the normal group, 1) P<0.05; compared with the model group, 2) P<0.05

2.3 Comparing the ratio of phospho-NF-κB p65/NF-κB p65 in uterus among groups

As can be seen from Figure 2 and Figure 3, the ratio of phospho-NF-κB p65/NF-κB p65 in the model group was significantly higher than that in the normal group (P<0.05); compared with the model group, the ratio of phospho-NF-κB p65/NF-κB p65 in the EA at acupoint group was significantly lower (P<0.05). There was no significant difference in the ratio of phospho-NF-κB p65/NF-κB p65 between the Western medicine group and the model group (P>0.05). Compared with the Western medicine group, the ratio of phospho-NF-κB p65/NF-κB p65 in the uterus of the EA at acupoint group was significantly lower (P<0.05). Compared with the normal group, the ratio of phospho-NF-κB p65/NF-κB p65 was significantly higher in the EA at non-acupoint group (P<0.05), and the difference was not statistically significant in the model group (P>0.05).

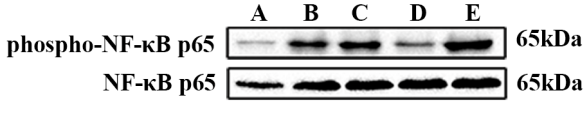

Figure 2. Western blot bands of phospho-NF-κB p65 and NF-κB p65 in rat uterus of each group

Note: A=Normal group; B=Model group; C=EA at non-acupoint group; D=EA at acupoint group; E=Western medicine group

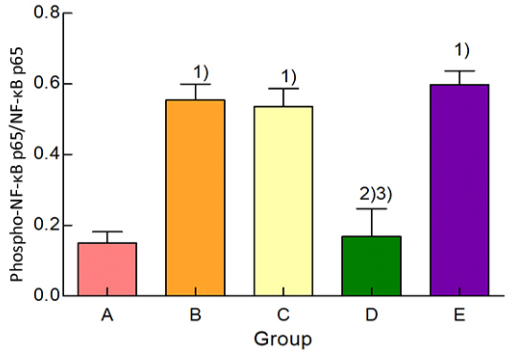

Figure 3. Comparing the ratio of phospho-NF-κB p65/NF-κB p65 in uterus among groups

Note: A=Normal group; B=Model group; C=EA at non-acupoint group; D=EA at acupoint group; E=Western medicine group; compared with the normal group, 1) $P<0.05$; compared with the model group, 2) $P<0.05$; compared with the Western medicine group, 3) $P<0.05$

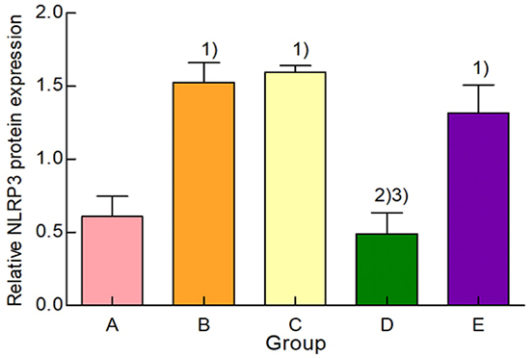

Figure 5. Comparison of NLRP3 protein expression in rat uterus among groups

Note: A=Normal group; B=Model group; C=EA at non-acupoint group; D=EA at acupoint group; E=Western medicine group; compared with the normal group, 1) $P<0.05$; compared with the model group, 2) $P<0.05$; compared with the Western medicine group, 3) $P<0.05$

2.4 Comparison of NLRP3, caspase-1, IL-1β and IL-18 protein expressions in rat uterus among groups

As can be seen from Figure 4-Figure 8, the NLRP3, caspase-1, IL-1β, and IL-18 protein levels in the model group were significantly higher than those in the normal group (all $P<0.05$). Compared with the model group, the protein levels of NLRP3, caspase-1, IL-1β and IL-18 in the EA at acupoint group were significantly lower (all $P<0.05$); compared with the model group, the protein levels of caspase-1, IL-1β and IL-18 in the uterine tissues of the Western medicine group were significantly decreased ($P<0.05$), however, the difference in the NLRP3 protein level was not statistically significant ($P>0.05$). Compared with the Western medicine group, the protein levels of NLRP3, IL-1β and IL-18 in the uterine tissues of the EA at acupoint group decreased significantly ($P<0.05$), but the difference in caspase-1 level was not statistically significant ($P>0.05$). Compared with the normal group, the NLRP3, caspase-1, IL-1β, and IL-18 protein levels were significantly increased in the EA at non-acupoint group (all $P<0.05$), while there was no significant difference in each protein level between the EA at non-acupoint group and the model group ($P>0.05$).

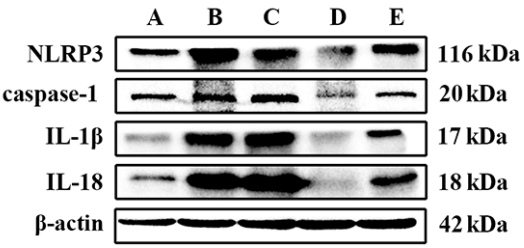

Figure 4. Western blot bands of NLRP3, caspase-1, IL-1β and IL-18 in rat uterus of each group

Note: A=Normal group; B=Model group; C=EA at non-acupoint group; D=EA at acupoint group; E=Western medicine group

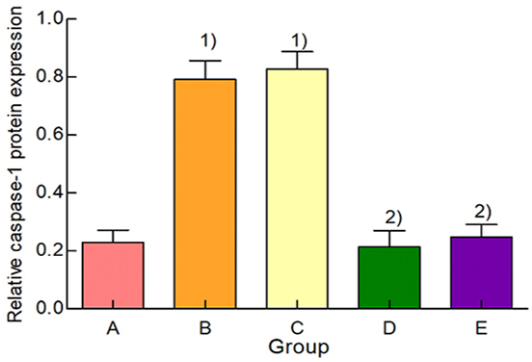

Figure 6. Comparison of caspase-1 protein expression in rat uterus among groups

Note: A=Normal group; B=Model group; C=EA at non-acupoint group; D=EA at acupoint group; E=Western medicine group; compared with the normal group, 1) $P<0.05$; compared with the model group, 2) $P<0.05$; compared with the Western medicine group, 3) $P<0.05$

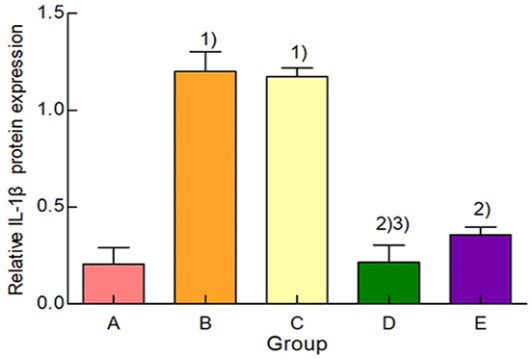

Figure 7. Comparison of IL-1β protein expression in rat uterus among groups

Note: A=Normal group; B=Model group; C=EA at non-acupoint group; D=EA at acupoint group; E=Western medicine group; compared with the normal group, 1) $P<0.05$; compared with the model group, 2) $P<0.05$; compared with the Western medicine group, 3) $P<0.05$

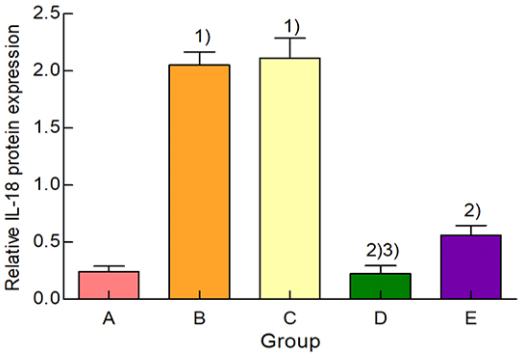

Figure 8. Comparison of IL-18 protein expression in rat uterus among groups

Note: A=Normal group; B=Model group; C=EA at non-acupoint group; D=EA at acupoint group; E=Western medicine group; compared with the normal group, 1) $P<0.05$; compared with the model group, 2) $P<0.05$; compared with the Western medicine group, 3) $P<0.05$

3 Discussion

PD is often affected by multiple pathogenic factors before, after or during menstruation, leading to blood stasis or cold accumulating in the Thoroughfare Vessel and the Conception Vessel, as a result, obstruction causes pain. It can also be caused by malnourishment due to liver/kidney deficiency and deficiency of qi and blood. Sanyinjiao (SP 6) is a crossing acupoint of three yin meridians of foot, and the liver, spleen and kidney are most closely related to dysmenorrhea. Guanyuan (CV 4) is where women store blood. The combination of the two acpoints can make the qi and blood smooth. Therefore, in the EA at acupoint group of this study, Sanyinjiao (SP 6) and Guanyuan (CV 4) were selected for EA treatment of PD model rats[14-17].

At present, many clinical studies have reported that acupuncture treatment of PD shows better short-term and long-term therapeutic effects, and the advantages of quick pain relief, good curative effect and no side effects have been found among the majority of patients[18-21]. The results of this study showed that both EA at acupoint and ibuprofen gavage method reduced the writhing numbers and improved the pathological damages of uteruses in PD rats, and the effects of these two were equivalent. There was no significant difference in any indicators between the EA at non-acupoint group and the model group. This indicated that EA had an analgesic effect on PD with acupoint specificity.

Previous studies have found that some pro-inflammatory factors such as IL-1β and TNF-α were significantly upregulated in the serum of PD patients[4], accompanied by neutrophil-based inflammatory cell infiltration in PD rat uterus sections[19], suggesting that inflammatory response plays an important role in the pathogenesis of PD. NF-κB is an important transcription factor for the expression of many inflammatory factors. When activated, NF-κB p65 becomes the active phospho-NF-κB p65, which promotes the expression and secretion of pro-inflammatory factors such as IL-1β and TNF-α, resulting in an aggravated inflammatory response[17]. Moreover, NF-κB is highly expressed in the uteruses of PD patients and rats[22-23]. The results showed that the ratio of phospho-NF-κB p65/NF-κB p65 in the model group was significantly higher than that in the normal group, which indicated that the phosphorylation level of NF-κB in PD was increased and NF-κB was activated. Compared with the model group, the ratio of phospho-NF-κB p65/NF-κB p65 in the EA at acupoint group was significantly decreased, which indicated that EA at acupoints reduced the NF-κB phosphorylation level and inhibited the NF-κB activation.

The NLRP3 inflammasome also mediates the regulation of IL-1β secretion. Activated NLRP3 inflammasome increases the pro-inflammatory factors such as mature IL-1β to aggravate the inflammatory response[6-8]. This study showed that the NLRP3, caspase-1, IL-1β and IL-18 protein levels in the model group were significantly increased, indicating that NLRP3 inflammasome activation, and IL-1β and IL-18 levels were upregulated in PD rats. The NLRP3, caspase-1, IL-1β and IL-18 levels of rat uteruses in the EA acupoint group were decreased, suggesting that EA at acupoint treatment may inhibit the production of inflammatory factors by negatively regulating the activation of NLRP3 inflammasome. In addition, this study also found that ibuprofen treatment had no significant effect on NF-κB p65 activation and NLRP3 protein expression in uteruses of PD rats, but reduced the protein expressions of caspase-1, IL-1β and IL-18.

In summary, this study suggests that EA at Sanyinjiao (SP 6) and Guanyuan (CV 4) has a positive effect on PD, which may be achieved through inhibition of NF-κB p65 phosphorylation and NLRP3 inflammasome activation, thereby effectively reducing the levels of pro-inflammatory factors IL-1β and IL-18.

Conflict of Interest

The authors declared that there was no potential conflict of interest in this article.

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Statement of Human and Animal Rights

The treatment of animals conformed to the ethical criteria.

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