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Electroacupuncture on Baihui (DU20) and Xuehai (SP10) acupoints alleviates psoriatic inflammation by regulating neurotransmitter substance P- Neurokinin-1 receptor signaling

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ABSTRACT

Background: At present, acupuncture-related practices have been widely used to treat psoriasis. In our study, we investigated the effect and explored the mechanism of electroacupuncture (EA) on acupoints Baihui (DU20) and Xuehai (SP10) for the treatment of psoriasis.

Methods: Imiquimod-induced psoriasis-like mouse model was used in this study. Mice were treated with electroacupuncture at DU20 and SP10 (depth of 2–3 mm, frequency of 2/15 Hz, intensity of 0.5–1.0 mA, 10 min/day). The severity of psoriasis-like lesions for each group was assessed. In addition, histological analysis of the lesions were performed. The levels of inflammatory cytokines were determined using Elisa. The expression levels of Substance P (SP) and NK1R were measured using Western blotting. In addition, NK1R inhibitor was administrated to evaluate the target of electroacupuncture in our mouse model.

Results: Electroacupuncture significantly alleviated IMQ-induced skin lesions and epidermal thickness, accompanied with reduced keratinocyte proliferation, $CD3^+$, $CD4^+$, and $CD8^+$ T cells infiltration. The reduced levels of inflammatory cytokines was observed after electroacupuncture treatment. In addition, electroacupuncture inhibited the expression levels of SP and NK1R. NK1R inhibitor could ameliorate lesional symptoms and suppress epidermal thickening and $CD3^+$, $CD4^+$, and CD8 + T cell infiltration.

Conclusions: Electroacupuncture relieved psoriasis-like inflammation and T cell infiltration. This therapeutic action was likely mediated by the modulation of Substance P and its receptor NK1R.

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1. Introduction

Psoriasis is a common, chronic, refractory, and inflammatory skin disease. The prevalence of psoriasis globally varies between 0.51% and 11.4%.[1] It is characterized by epidermal hyperplasia, dilated and prominent blood vessels in the dermis, and cutaneous immune cell infiltration, including T cells, dendritic cells, and neutrophils.[2] The incidence of psoriasis has increased significantly in the past few years. The IL-23/Th17 cell axis has been shown to play an essential role in its pathogenesis.[3] Numerous publications have reported the critical role of neurogenic inflammation in the pathogenesis of psoriasis.[4,5] The symptoms of IMQ-induced

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List of abbreviations

EA	Electroacupuncture
DU20	Baihui
SP10	Xuehai
SP	Substance P
NK1R	Neurokinin-1 receptor signaling
IMQ	Imiquimod
MTX	Methotrexate
DU14	Dazhui
ST36	Zusanli
RIPA	Radio Immunoprecipitation Assay
PVDF	Polyvinylidene fluoride
IL- 27	Interleukin 17A
IFN-γ	Interferon-γ
IL-22	Interleukin 22
IL-23	Interleukin 23
IL-12p70	Interleukin 12p70
TNF-α	Tumor necrosis factor-alpha
IL-1β	Interleukin1β
IL-9	Interleukin 9
IL-6	Interleukin 6
IL-18	Interleukin18
MK869	Aprepitant (MK-0869, L-754030)
HE	Hematoxylin & Eosin

psoriasis can be alleviated by skin denervation or by using neurotransmitter inhibitors.[6–8] These results suggest that the nervous system plays a significant role in psoriasis pathogenesis.

The skin is a highly complex organ composed of the epidermis, dermis, and hypodermis and is responsible for sensation and protection against environmental pollutants, foreign proteins, and infection.[5] The skin is considered a sensory organ and an essential part of the central nervous system.[9] Infiltrating cells in the skin respond to stimuli by synthesizing and releasing chemical mediators, such as hormones, neurohormones, cytokines, neuropeptides, and neurotransmitters.[10] The binding to their receptors induces the signaling and activation of the immune and nervous systems. This triggers the interaction between skin cells, immune cells, and skin nerve fibers.[11] Studies have shown that neuropeptide SP is significantly increased in the lesions of patients with psoriasis.[12] SP activates the inflammatory reaction, which results in T-lymphocyte proliferation and mast cell degranulation during the initial stages of psoriasis.[13,14]

Electroacupuncture, a traditional therapy, has been recommended as a complementary therapy for psoriasis. In our previous study, we compared acupuncture, electroacupuncture, and fire acupuncture. We found that electroacupuncture of the Dazhui (DU14) and the right Zusanli (ST36) acupoints had the best therapeutic effects.[15] Before this study, we systematically reviewed published clinical reports for treating psoriasis using acupuncture and moxibustion. Based on this review, we selected the most common acupoints used, i.e., DU20 and SP10. In this study, we used the IMQ-induced psoriasis-like mouse model to determine whether electroacupuncture on DU20/SP10 could regulate the expression of local substance P in the skin and play a role in alleviating T cell infiltration and improving skin inflammation.

2. Materials and methods

2.1. Animals

C57BL/6 (male, eight weeks) mice were purchased from the Beijing Huafukang Bioscience Company (animal license number SCXK, 2019–0008, Beijing) and housed under SPF conditions, controlled temperature (22–24 °C), and 12h light/dark cycle, with free access to food and water.

All animal experiments were approved by the Animal Ethics Committee of the Beijing Institute of Traditional Chinese Medicine (No.2019070201). All animal experiments met the requirements of the National Institutes of Health Guidelines on Laboratory Research. Every effort was made to minimize the number of animals used and reduce animal suffering.

2.2. Model preparation and treatment groups

2.2.1. Experiment I

After a week of adaptive feeding, the hair on the back of each mouse was shaved (approximately 2 cm × 3 cm). Surgeries were performed under anesthesia. Mice were randomly divided into assigned groups and housed in single cages. Mouse models were randomized into four groups: (1) blank control (Ctrl, n = 8): Vaseline was smeared on the notum for seven days; (2) Usage Model Workgroup (IMQ, n = 8), 62.5 mg of 5% Imiquimod cream (Ming Xin Pharmaceutical Co. Ltd, China) was smeared on the notum of mice for seven days. Ctrl and IMQ groups were intragastrically administered 0.2 ml saline daily from day 4 for three days; (3) Methotrexate group (MTX, Shanghaixinyi Pharmaceutical Co.Ltd., China). MTX was dissolved in distilled water to a final concentration of 1 mg/kg 62.5 mg of 5% Imiquimod cream was applied on the notum of mice for seven days. From day 4, MTX was administered intra-gastrically at a dose of 1 mg/kg/d for three days; (4) the electroacupuncture group (EA, n = 8): Imiquimod cream was smeared for seven days, after which electroacupuncture was performed at the DU20 and right SP10 acupoints for 10 min. Based on a previous study, the location of DU20 acupoint in mice was 3 mm lateral to the midpoint of a line joining the two ears at the back of the head. The SP10 acupoint is located in the depression anterior and distal to the head of the fibula of mice. Two stainless steel acupuncture needles (diameter, 0.25 mm; length, 25 mm; Suzhou Medical Appliance Factory, Suzhou, China) were inserted at the DU20 single acupoint at 0.5–1 mm depth in the direction of the opposite eye (Fig. 1). The top end of the needle handle was then connected to an electrical stimulator (Han's acupuncture point nerve stimulator HNAS-200E; Nanjing, China) for 10 min/day. This was performed on the fourth day continuously for three days. The EA stimulator was set at a 2/100 Hz (Dilatational wave) frequency and an intensity of 0.5–1.0 mA.

2.2.2. Experiment II

After a week of adaptive feeding, the hair on the back of each mouse was shaved (approximately 2 cm × 3 cm). All surgeries were performed under anesthesia. Mice were randomly divided into assigned groups and housed in single cages. Mouse models were randomized into five groups: (1) Ctrl group (n = 8): Vaseline was applied on the notum for seven days; (2) IMQ group (n = 8), 62.5 mg of 5% Imiquimod cream was applied on the notum of mice for seven days. Ctrl and IMQ groups were intra-gastrically administered 0.2 ml saline daily from day 4 for three days; (3) EA group (n = 8): Imiquimod cream was applied for seven days. Electroacupuncture treatment was similar to Experiment I; (4) IMQ + MK-0869 group (n = 8); 62.5 mg of 5% Imiquimod cream was applied on

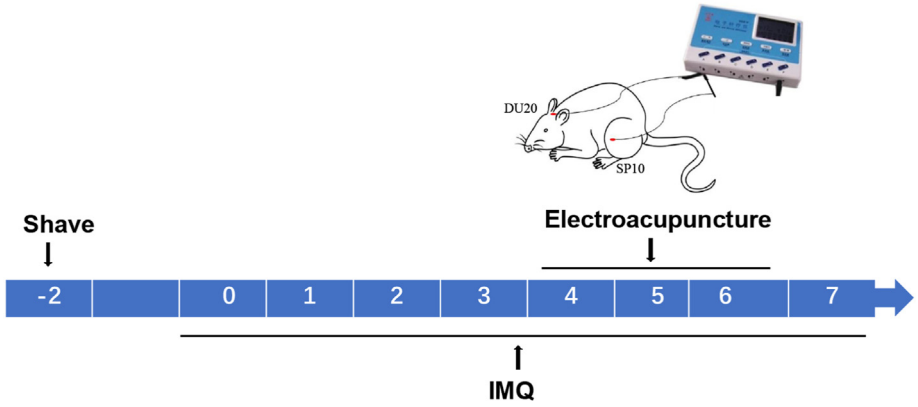

Fig. 1. Diagram of the experimental protocol. Except for the Ctrl group, all other groups were treated topically with 62.5 mg IMQ cream (5%) on days 1–6. Electroacupuncture treatment was performed daily from day 4–6 for the electroacupuncture group.

the notum of mice for seven days and intra-gastrically administered at 0.2 ml MK-0869(4 mg/kg/d) daily from day 4 for three days; (5) EA + MK-0869 group (n = 8), 62.5 mg of 5% Imiquimod cream was applied on the notum of mice for seven days. From day 4, mice were subjected to electroacupuncture and intragastric administration of MK-0869 at 4 mg/kg/d for three days.

For the preparation of MK-0869 type solution. MK-0869 was dissolved in the vehicle (5% DMS+40% PEG300 + 5% Tween 80) to a final concentration of 4 mg/kg.

2.3. Establishment of the psoriasis-like mouse model and severity score analysis

The control group was administered Vaseline to the shaved area on the back. The other groups were treated topically with 62.5 mg IMQ cream (5%) (Mingxinlidi Laboratory, China) for seven days. Scale, thickness, and erythema were rated daily on a scale of 0–4 according to the PASI scoring criteria: 0, none; 1. mild. 2. moderate; 3. severe; and 4, very severe. The total scores for scale, thickness, and erythema were calculated, and the overall trend for PASI scores was plotted.

2.4. Histology, immunohistochemistry, and immunofluorescence staining

Histological staining was performed: 5 μm paraffin sections from psoriatic mouse skin were HE stained. Histological changes were observed and photographed under a light microscope (Axio Imager, M2, Zeiss, Germany). Immunohistochemistry and immunofluorescence staining were performed as follows: Tissue sections were stained with anti-Rabbit CD3 (1:100, Abcam, USA), anti-Rabbit CD4 (1:1000, Abcam, USA), anti-CD8 antibody (1:2000, Abcam, USA), anti-SP antibody (1:500, Abcam, USA) and Ki-67 (1:400 dilution; Abcam, Cat. Ab15580, USA). Images were obtained using a light microscope and fluorescence microscopes to determine expression levels.

2.5. Elisa

Approximately 0.5–0.8 ml of blood was collected by cardiac puncture under ketamine and incubated for 30 min at room temperature. The blood was then centrifuged at 3000 rpm, 4 °C for 15 min and the resulting serum was stored at − 80 °C. The levels of cytokines IL-17A, IL-23, IL-22, IL-6, TNF-α, and IL-1β in the serum samples were determined using an ELISA kit (LAIZEE, China) according to the manufacturer's instructions. The absorbance was read at 450 nm using a microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific, USA).

2.6. Western blotting

Total protein from skin tissues was extracted using RIPA buffer containing phosphatase and protease inhibitors. Lysates were then centrifuged at 12,000 g for 10 min. Protein concentrations were measured using the Pierce BCA Protein Determination Kit (Cat. No.23227, Thermo Scientific, USA). 50 μg of total protein/well were then separated using a 10%/12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto PVDF membranes. PVDF membranes were blocked in 5% skim milk for 1 h at room temperature and then incubated with primary antibodies against GAPDH (1:5,000, ImmunoWay Biotechnology Company, USA), SP (1:1000, Cell Signaling Technology, USA); or NK1R (1:2000, Cell Signaling Technology, USA) overnight at 4 °C. Membranes were then washed with Tween 20 wash buffer and then incubated with secondary anti-rabbit IgG (H + L) (111-035-003, Jackson Immuno Research Laboratories, West Grove, PA, USA; 1:10,000) or goat anti-mouse IgG (H + L) (115-035-003, Jackson ImmunoResearch Laboratories; 1:10,000) at room temperature for 1 h. Immunofluorescence was measured using an Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE, USA). Specific protein bands were quantified using the ImageJ one analysis software.

2.7. Statistical analyses

GraphPad Prism 6.0 was used for statistical analysis and the generation of plots. All measurement data were expressed as mean ± standard deviation (SD). Two groups were compared using a *t*-test, and multiple groups were compared using a one-way analysis of variance (ANOVA). P-values less than 0.05 were considered statistically significant.

3. Results

3.1. Electroacupuncture improves psoriasis-like lesions in IMQ-Induced Mice

We assessed whether electroacupuncture had a therapeutic effect on psoriasis. The IMQ-induced psoriatic mouse model was treated with electroacupuncture at DU20 and SP10 acupoints, as described in Fig. 1A. Control mice (no IMQ administration) did not show signs of skin inflammation. Compared to control mice, mice treated topically with IMQ for three days showed signs of psoriasis-like lesions, erythema, scaling, and skin thickening. Mice were

administered electroacupuncture or methotrexate (MTX) from day four onwards. After three days of treatment, electroacupuncture and MTX attenuated the skin lesions of psoriatic mice remarkably. However, the model group mice showed severe symptoms of psoriasis-like skin lesions, including skin erythema, scales, and skin thickness, after seven days of IMQ treatment. The PASI scores of electroacupuncture-treated or MTX-treated mice were significantly lower than those in the IMQ group on days 5–7 (Fig. 2A and B). Epidermal hyperplasia is one of the typical pathological characteristics of psoriasis. H&E staining showed that after seven days of IMQ administration, mice in the model group had significant epidermal hyperplasia and parakeratosis. This was alleviated after treatment with electroacupuncture or MTX. Our results demonstrated that electroacupuncture reduced the skin lesions in IMQ-induced psoriatic mice and significantly reduced epidermal thickness ($P < 0.001$) (Fig. 2C and D).

3.2. Electroacupuncture alleviates keratinocyte proliferation and inflammatory cell infiltration in psoriatic mice

Ki67 is a nuclear antigen associated with cell proliferation.[16] The nuclei of positive cells are shown by green fluorescence. In the Ctrl group, several good spots were distributed in the epidermal basal layer. Compared to the Ctrl group, the number of positive cells in the IMQ group increased significantly ($P < 0.01$). Compared to the model group, the number of positive cells in the methotrexate and electroacupuncture groups was significantly reduced ($P < 0.01$) (Fig. 3A and E). We then measured the impact of EA on inflammatory cell infiltration. Immunohistochemistry staining showed an increase in the infiltration of CD3+/CD4+/CD8+ T cells was observed in IMQ-induced lesions but was reduced in the MTX, and EA treated groups (Fig. 3B-D and 3F-H).

3.3. Electroacupuncture reduces the secretion of inflammatory cytokines in the serum of psoriasis-like mouse models

Next, we measured the secretion of several inflammatory cytokines in mouse serum. Compared to the control group, the levels of IL- 27, IFN-γ, IL-22, IL-23, IL-17A, IL-12p70, TNF-α, IL-1β, IL-9, IL-6, and IL-18 were significantly increased in the serum of mice in the model group ($P < 0.05$). IL-27, IL-22, IL-17A, IL-1β, IL-9, and IL-6 were remarkably reduced in the EA group compared to the model group ($P < 0.05$). IL-27, IFN-γ, IL-23, IL-12P70, TNF-α, and IL-18 showed a decreasing trend but no statistical significance. In addition, the levels of IL-27, IL-17A, TNF-α, IL-1β, and IL-6 were reduced in the MTX group ($P < 0.05$). These results indicated that EA suppressed the secretion of inflammatory cytokines in the serum of psoriasis-like mouse models (Fig. 4A–K).

3.4. Electroacupuncture inhibits the levels of neurotransmitter SP and its receptor NK1R in imiquimod-induced psoriasis-like mouse models

Recent studies have demonstrated that sensory neurogenic polypeptides result in psoriasiform dermatitis.[17] SP is a member of the neuropeptide family and is abundant in the central nervous system and surrounding tissues. It participates in neural activity, immune regulation, and inflammatory response. Hence, we measured the expression of neuropeptides in mouse skin. Immunofluorescence staining showed that the expression of SP in the dermis was higher compared to mice in the IMQ group and was reduced in the EA group (Fig. 5A). NK1R is a high-affinity receptor for SP, and the SP-NK1R complex is the molecular basis for several pathological processes.[18] We measured the protein expression levels of SP and NK1R on the 7th day after acupuncture by western blotting. Compared to the control group, the expression levels of

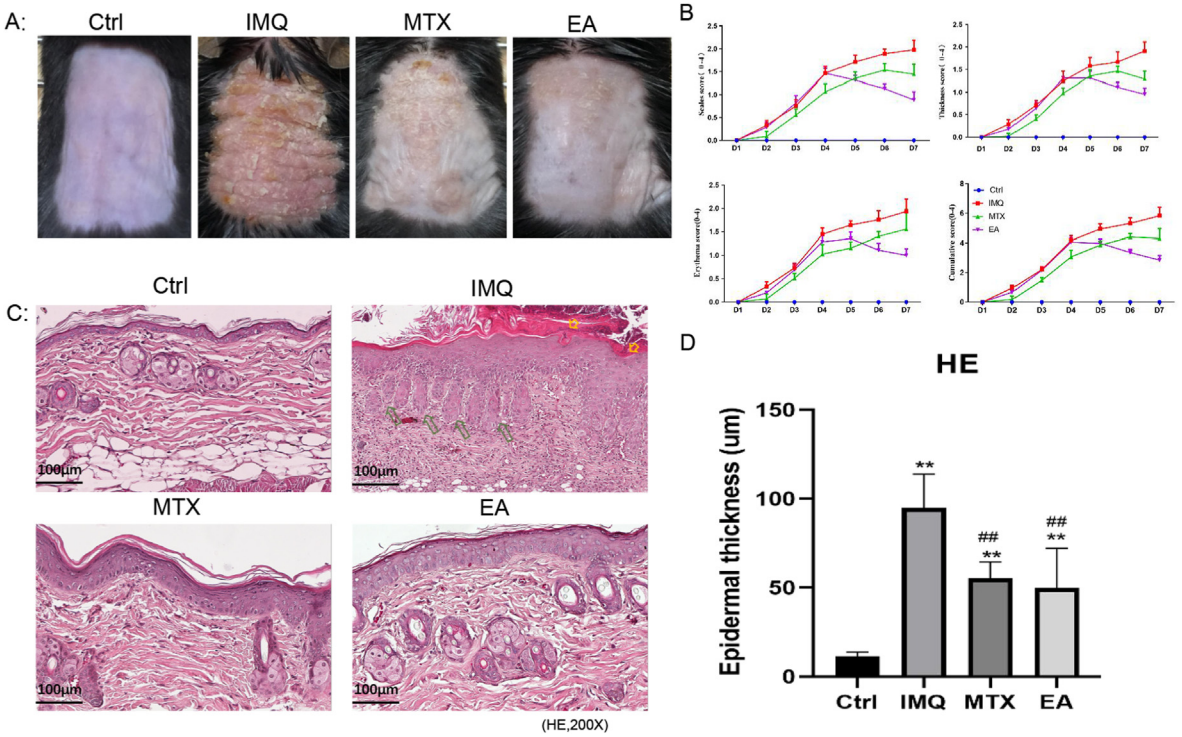

Fig. 2. Electroacupuncture ameliorates psoriasis-like inflammation in IMQ-Induced Mice. (A) Skin lesions in mice on day seven after IMQ administration. (B) Psoriasis Area Severity Index (PASI) scores for skin damage, i.e., scaling, thickness, and erythema. Cumulative score (scaling + thickness + erythema). (C) HE at 200 × . (D) The epidermal thickness of the dorsal skin. Scale bar = 50 μm. Data are presented as the mean ± SD (n = 8/group). ** <0.01 vs Ctrl; # <0.05vs IMQ, ## <0.01 vs IMQ.

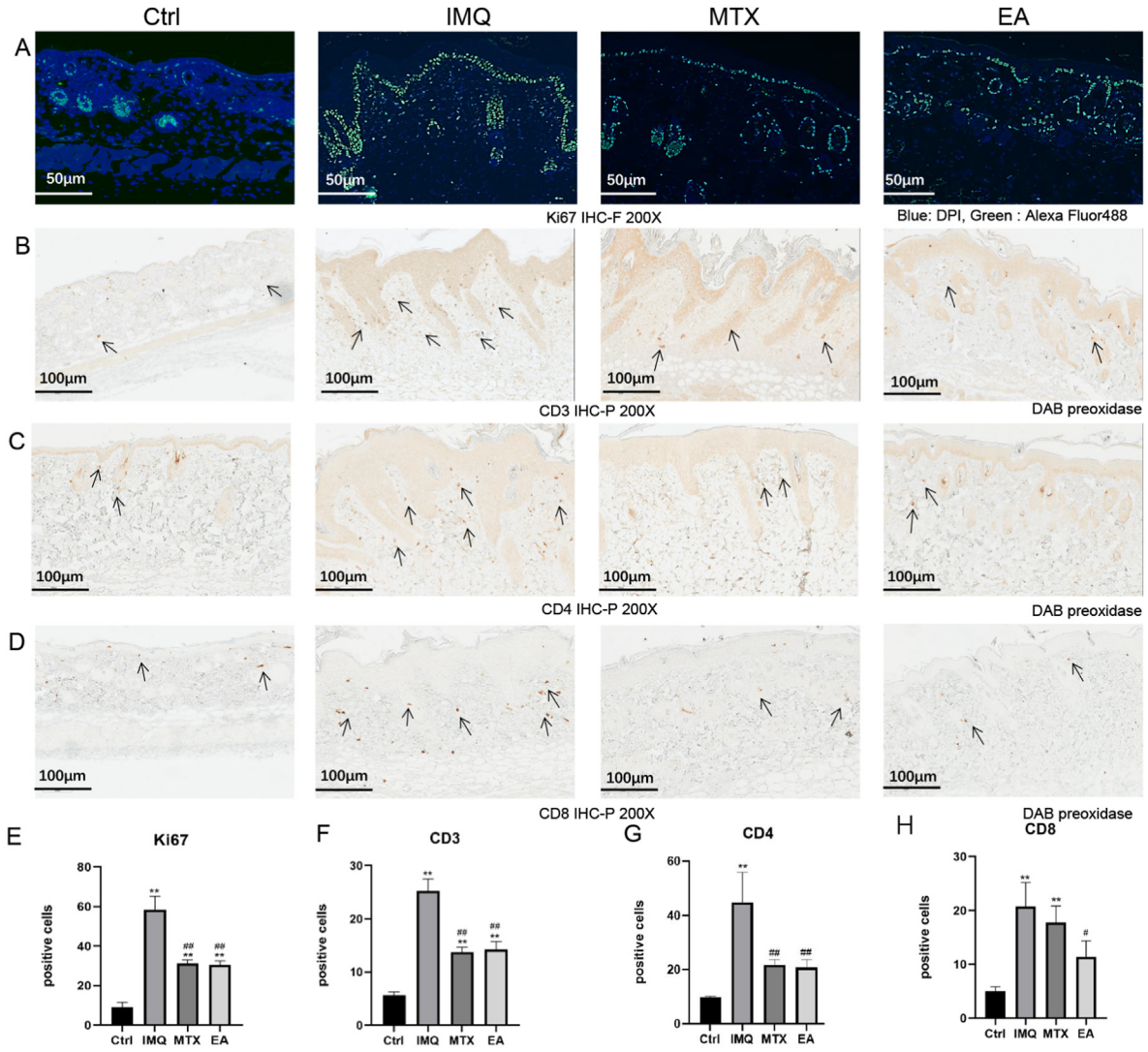

Fig. 3. Electroacupuncture alleviates keratinocyte proliferation and inflammatory cell infiltration in psoriatic mice. (A) Expression of Ki67 in the skin cells of mice for each group (IF) Scale bar = 50 μm. (B–D) IHC staining for CD3, CD8, and CD4. (E–H) Statistical analysis of the number of Ki67+, $CD3^+$, $CD4^+$, and $CD8^+$ cells in the epidermis. Data are presented as mean ± SD (n = 5/group). Scale bar = 200 μm ** <0.01 vs Ctrl; # <0.05vs IMQ, ## <0.01 vs IMQ.

SP/NK1R in the IMQ group were significantly increased but were reduced in the EA group (Fig. 5B and C). This demonstrates that EA could inhibit the expression levels of local neurotransmitters from preventing the activation of local neurotransmitter receptors.

3.5. Correlation analysis between expression levels of inflammatory cytokines and SP

Pearson's correlation determined the relationship between SP protein expression levels and proinflammatory cytokine IL-17A and IL-22 secretion in skin lesions on the 7th day (n = 3). Our results demonstrated that IL-17A and IL-22 were positively associated with SP expression levels. Compared to the Ctrl group, the expression levels of inflammatory factors and SP were significantly increased in the IMQ group but were reversed in the EA group. (Fig. 6A and B).

3.6. Electroacupuncture and NK1R inhibitors reduced IMQ-induced skin thickening and inflammatory cell infiltration

SP and NK1R have essential roles in the pathogenesis of IMQ-induced psoriasiform lesions. We investigated whether NK1R inhibitors (MK-0869) could improve the pathological manifestations and inflammatory cell infiltration observed in psoriatic lesions. After treatment with MK-0869, H&E staining demonstrated that the epidermis was reduced in thickness compared to mice in the IMQ group (Fig. 7A and E). In addition, compared to the IMQ group, inflammatory CD3/CD4/CD8 T-cell infiltration was reduced significantly after MK-0869 treatment (Fig. 7B–D and F-H). When MK-0869 was administered in combination with EA, the ability of EA to ameliorate psoriasiform lesions and CD3/CD4/CD8 T-cell infiltration was absent. Our results suggest that SP and NK1R are critical regulators for EA efficacy in psoriasis.

4. Discussion

We investigated the effects and mechanism of EA administration at DU20 and SP10 acupoints for treating psoriasis. IMQ-induced psoriasis-like mouse models were generated using C57 BL/6 mice. We observed that EA significantly reduced the severity of skin lesions (erythema, scaling, epidermal thickness, and inflammatory cell infiltration) in our psoriasis-like mouse model. EA treatment attenuated IMQ-induced secretion of inflammatory

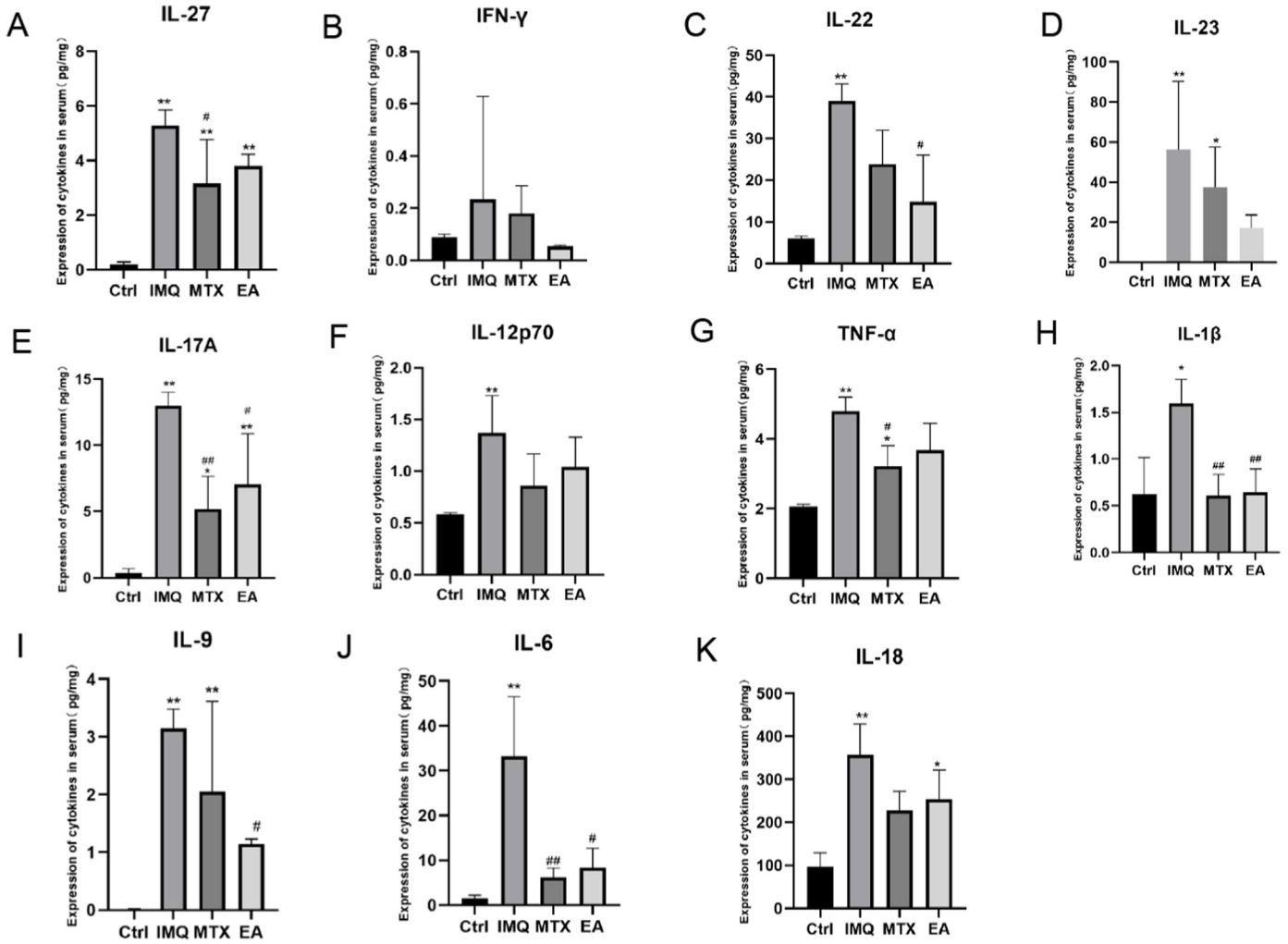

Fig. 4. Electroacupuncture reduced the expression levels of inflammatory cytokines in the serum of psoriasis-like mouse models. Data are presented as mean ± SD(A-K) n = 5/group. *<0.05 vs Ctrl, ** <0.01 vs Ctrl; # <0.05vs IMQ, ## <0.01 vs IMQ.

cytokines, including IL-27, IL-22, IL-1β, IL-9, IL-6, and IL-17A in the serum. This therapeutic action is mediated by the modulation of Substance P and its receptor NK1R. (Fig. 7).

In recent years, acupuncture-related practices have been widely used to treat psoriasis. These include acupuncture, moxibustion, cupping therapy, ear auricular pressure treatment, acupoint catgut embedding therapy, blood puncture therapy, acupuncture point injection therapy, fumigation treatment with Chinese medicines, fire needling therapy, and electroacupuncture.[19–21] Our previous study demonstrated the efficacy of acupuncture, electro-acupuncture, and fire acupuncture on psoriasis. Electroacupuncture was found to have the best therapeutic effect.[15] DU20/SP10 are the most common acupuncture points that have been used in clinical trials and practice to treat psoriasis.[19] Hence, we selected DU20/SP10 acupoints in this study to treat psoriasis and investigate the underlying mechanisms.

Psoriasis is a complicated, inflammatory skin disease mediated by various cells. These include keratinocytes, T cells, endothelial cells, macrophages, and dendritic cells. T-cell activation results in keratinocyte proliferation and is thought to be the main pathogenic mechanism of psoriasis.[22] After acupuncture treatment, PASI scores, IL-22 and IL-17, and $CD8^+$ and $CD4^+$ were reduced significantly in patients.[23]

The IMQ-induced psoriasis-like skin mouse model was used in this study to investigate the effects of EA on the inflammatory response and T cell activation. We found that erythema and scaling were induced after IMQ. Compared to the IMQ group, mice in the EA group showed an improvement in skin lesions. The severity of skin lesions (erythema, scaling) was scored on days 1~7 based on PASI. We observed that mice treated with EA had a lower score (erythema, scaling) than the IMQ group on day 7. Histopathological analysis showed that the epidermal thickness was significantly reduced after EA treatment. We next investigated the effects of EA on the inflammatory response of T cell activation. Immunohistochemistry demonstrated that EA reduced $CD3^+$, $CD4^+$, and $CD8^+$ T cell numbers in skin lesions. Flexible Multilyte Profiling showed the levels of inflammatory cytokines, i.e., IL- 27, IFN-γ , IL-22, IL-23, IL-17A, IL-12p70, TNF-α, IL-1β, IL-9, IL-6, and IL-18 were increased in the IMQ group. EA treatment attenuated Th1- and Th17-type cytokine expression levels, including IL-1β, IL-22, IL-17A, IL-27, and IFN-γ. Interleukin-27 (IL-27) is a member of the IL-12 family. IL-27 is produced by antigen-presenting cells (APCs), including dendritic cells (DCs), monocytes, and macrophages. It has been proposed that IL-27 has a dual role in the pathogenesis of psoriasis. IL-27 promotes the onset of psoriasis by inducing chemokines in keratinocytes, but it may also inhibit inflammation by suppressing TNFα-induced cytokines and chemokines. Compared to control mice injected with a vehicle, IL-27 administration increased mRNA

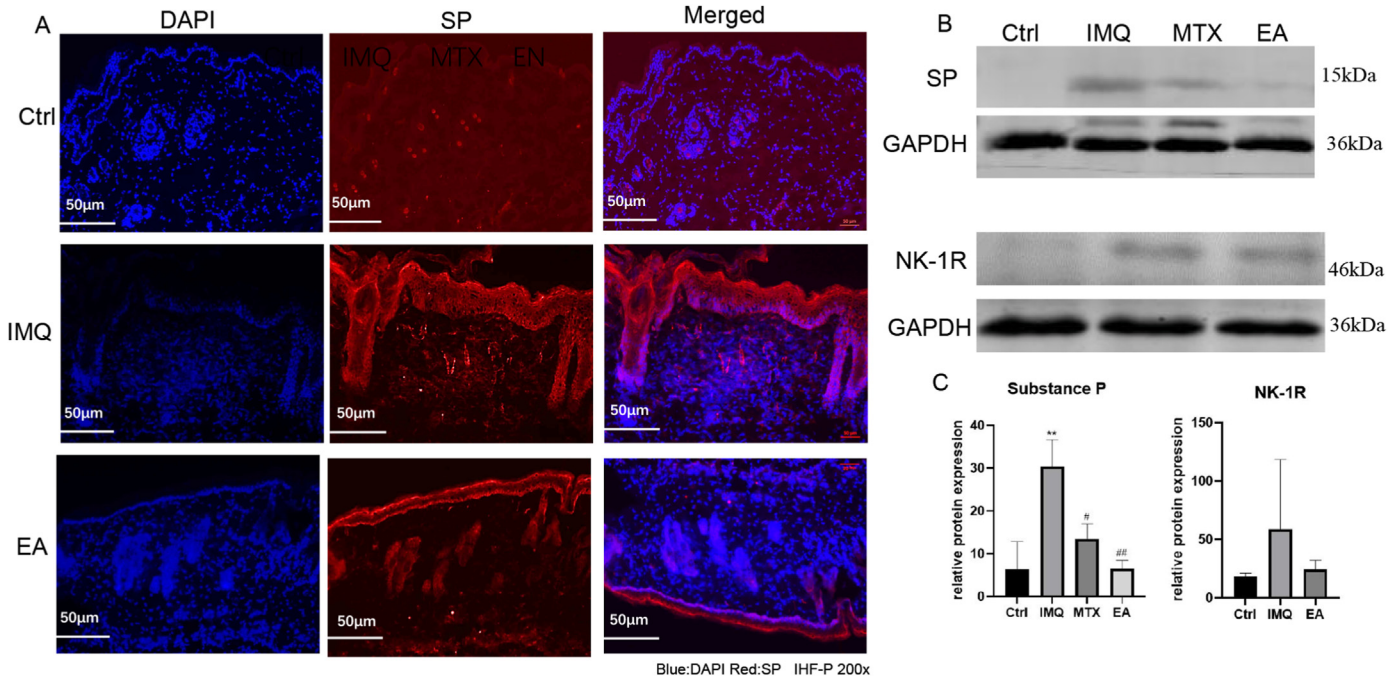

Fig. 5. Electroacupuncture inhibits the expression levels of neurotransmitter SP and its receptor NK1R in imiquimod-induced psoriasis-like mouse models. (A) Immunofluorescence staining of SP.(B) Representative immunoblots of SP and NK1R by western blot. (C) Relative protein expression levels and statistical analysis for SP and NK1R levels. Data are presented as mean ± SD (n = 4/group). *<0.05 vs Ctrl, ** <0.01 vs Ctrl; # <0.05vs IMQ, ## <0.01 vs IMQ.

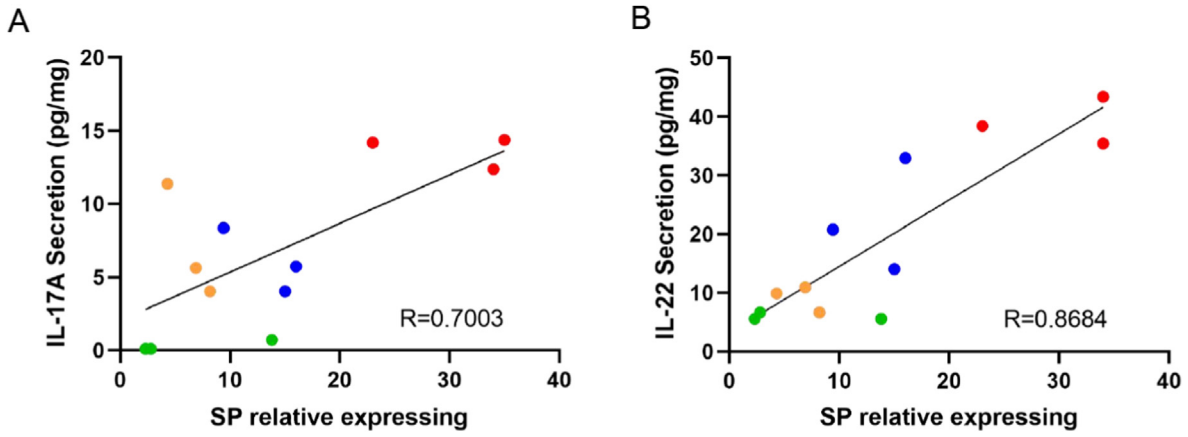

Fig. 6. Correlation analysis between expression levels of inflammatory cytokines and SP. A: IL17A vs SP; B: IL-22 vs SP. R: Pearson's correlation. (Green: Ctrl, Yellow: EN, Blue: MTX, Red: IMQ)

levels for IFN-γ, TNFα, and various chemokines in the injected skin, exacerbating psoriasis-like skin lesions in mice. However, the levels of inflammation were reduced after neutralization of the IL-27 effect.[24] IL-9 and IL-6 can induce Th17 cells to differentiate and enhance the production of IL-17 from Th17 cells in vitro.[25,26] These results demonstrated that EA could improve imiquimod-induced psoriasis-like skin lesions by reducing T cell infiltration and inflammatory cytokines.

Psoriasis is a complex, multifactorial disease whose pathogenesis has not been fully elucidated. Studies investigating the distribution of cutaneous nerves and the quantification of nerve growth factor and neuropeptides in lesioned and non-lesioned psoriatic skin have suggested that sensory neuropeptides contribute to the development of psoriasis.[27,28] It has been demonstrated that the number of substance P and NK1R immunoreactive cells were more involved than non-involved psoriatic skin17. Similarly, B. Amatya et al. found that intradermally injected substance P induced pruritus, flares, and wheal in psoriasis patients.[29] However, these responses did not differ significantly from those of healthy controls. In recent years, numerous publications have reported that psoriatic patients had spontaneous clearance or conditional improvement in their skin lesions after acquired central or peripheral nerve damage.[30] Similarly, cutaneous denervation by traumatic nerve injury reduced inflammatory manifestations in psoriatic mice8. These results suggest that the nervous system may contribute to the pathogenesis of psoriasis.

Substance P (SP) is a highly conserved peptide first discovered by Von Euler and Gaddum in 1931 in horse brain and intestinal extracts.[31] SP is encoded by the TAC1 gene (located on human chromosome 7) and is a member of the tachykinin hormone family 32. SP is released by peripheral and central nerve endings of sensory neurons in the central nervous system (CNS). In addition, it is

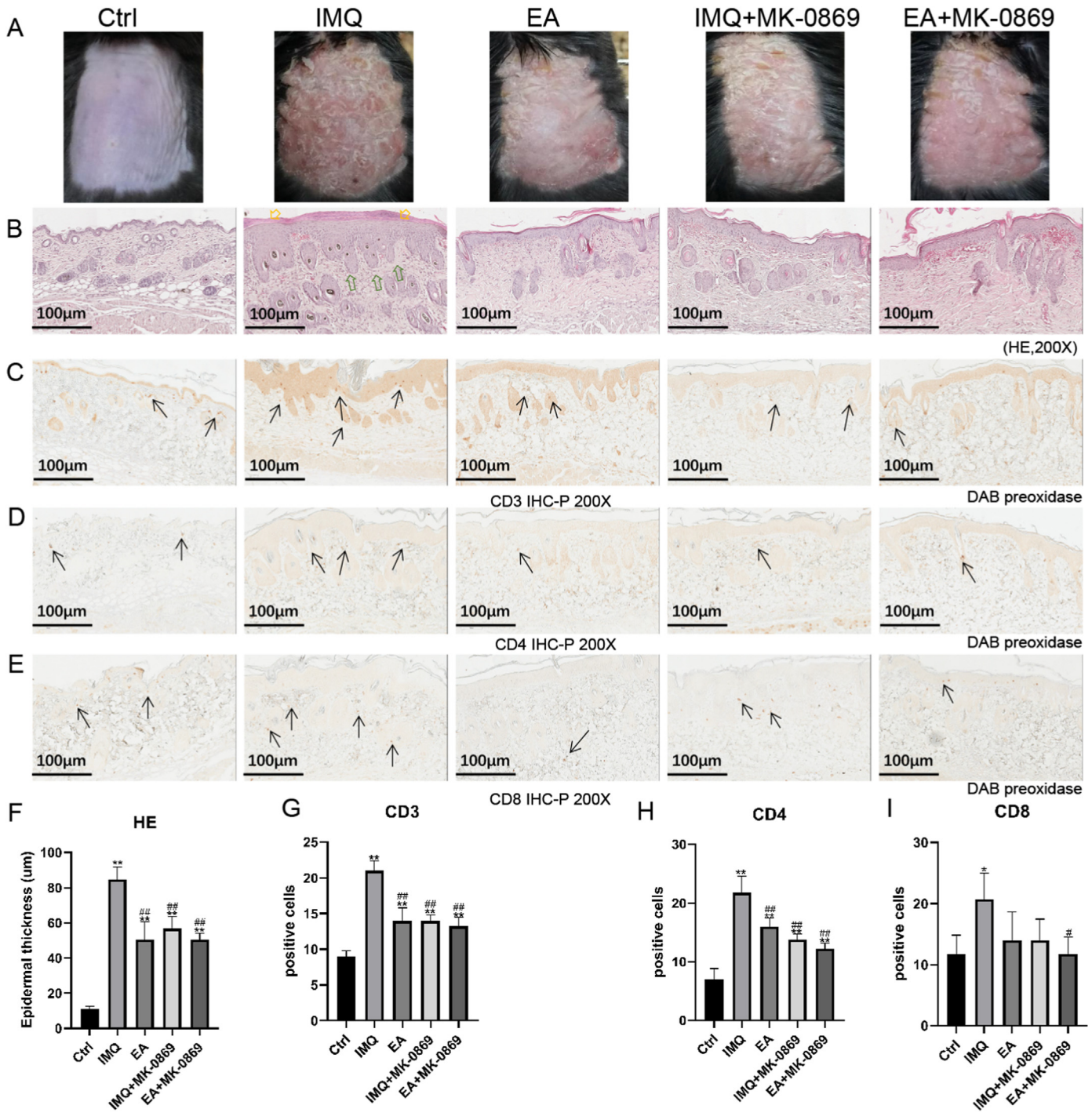

Fig. 7. Electroacupuncture and NK1R inhibitors reduced IMQ-induced skin thickening and inflammatory cell infiltration. (A) Skin lesions in mice on day 6 of IMQ administration. (B) HE staining (200 ×), Scale bar = 100 μm. (C–E) IHC staining for CD3, CD8, and CD4. Data is presented as mean ± SD (n = 3–4/group) Scale bar = 100 μm* <0.05 vs Ctrl, ** <0.01 vs Ctrl; # <0.05 vs IMQ, ## <0.01 vs IMQ. (F) Epidermal thickness for each group. (G–I) Statistical analysis of the number of $CD3^+$, $CD4^+$, and $CD8^+$ cells in the epidermis.

secreted by a variety of cells of the immune system.[32] NK1R is a widely expressed G-protein coupled receptor found in neuronal and non-neuronal cells. A recent publication described a truncated isoform (NK1R-T) found in monocytes and other peripheral cells.[33,34] SP is a sensory neurotransmitter that is associated with skin sensation. In the skin, SP is predominantly expressed at the dermo-epidermal junction and less frequently in the intra-epidermal area.[35,36] SP recognizes NK1R on T cells and induces the production of IFN-γ.[37] SP and HK-1 have been shown to shift non-Th17-committed CD4(+) memory T cells into bona fide Th17 and Th1/Th17 cells. SP- and HK-1-induced Th17 cell generation is mediated through NK1R.[38] SP and HK-1 trigger IL-1β, IL-6, and TNF-α production to upregulate IL-23 production. SP plays an essential role in promoting the production of inflammatory cytokines by T cells, which can themselves produce more SP and NK1R and stimulate antigen-presenting cells to produce additional T cell stimulatory cytokines. This leads to an up-regulation of Th17 and Th1 responses.[39–41] SP promotes skin inflammation by inducing

the expression of nerve growth factors and leukotriene B4 in keratinocytes and release histamine from mast cells. In addition, SP stimulates mast cells to secrete IL-1 family cytokines, which in turn stimulates keratinocyte proliferation. These findings suggest that blocking SP and its receptor could have beneficial effects on psoriasis. Several studies have shown that electroacupuncture alleviates inflammatory diseases by inhibiting SP expression.[42–44] In the present study, we demonstrated by western blot analysis and immunofluorescence assays, that expression levels of SP were higher in IMQ - induced psoriatic lesions. We found that electroacupuncture was able to decrease the expression levels of SP and Neurokinin 1 receptors (NK1R). To validate our findings, IMQ-induced psoriasis-like mouse models were administered an NK1R inhibitor Aprepitant (MK-0869) resulting in reduced skin lesions. H&E staining demonstrated that the epidermis of MK-0869-treated mice had reduced thickness compared to mice in the IMQ group. In addition, MK-0869 partially reduced $CD3^+$/CD4+ and $CD8^+$ T cell infiltration. The reduction in epidermal thickness and the levels of infiltrating $CD3^+$/CD4+ and $CD8^+$ T cells was similar between MK-0869- and EA-treated mice when compared to mice in the IMQ group. Giovanni Damiani et al. suggested that Aprepitant (high-affinity NK1R antagonist) may be a good alternative for the treatment of Psoriasis-related pruritus in patients under antihistamine administration.[45] When MK-0869 was administered together with EA, the ability of EA to ameliorate psoriasiform lesions and CD3/CD4/CD8 T-cell infiltration was negated. Based on these findings, we believe that SP and NK1R are critical for the efficacy of EA in the treatment of psoriasis.

In conclusion, electroacupuncture stimulation at DU20 and SP10 acupoints relieved IMQ -induced psoriasis inflammation and T cell infiltration in mice. This beneficial action is most likely mediated via the modulation of Substance P and its receptor NK1R.

5. Limitations of the article

We found that electroacupuncture at DU20 and SP10 was beneficial in reducing inflammatory responses. However, the pathway through which SP interacts with NK1R to signal various intracellular pathways warrants further investigation. These pathways involve inositol trisphosphate (IP3), diacyl-glycerol (DAG), and cyclic adenosine monophosphate (cAMP), which control the expression of cytokines. SP-NK1R coupling induces adenylate cyclase and phospholipase C to generate DAG/IP3 and cAMP and then transmits signals to mitogen-activated protein kinases (MAPKK or MEK). MEKs activate extracellular signal-associated kinase 1/2 (ERK1/2), which is transported to the nucleus by transcription factors such as serine/threonine-protein kinase and mammalian target of rapamycin (mTOR) to mediate cytokine expression.[46] However, the signaling pathway at the skin lesions was not assessed and needs to be investigated in future studies.

Data availability

All data generated or analyzed in this study are included in this article.

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Authors’ contribution

Cong Qi: Conceptualization, Methodology, Software, Collating of data, Compose the original draft. Fang Feng: Collating of data, Compose the original draft. JianNing Guo:Collating of data. Yu Liu: Investigation,Visualization. Xiaoyao Guo: Collating of data, Plot.Yujiao Meng: Collating of data, Plot. Tingting Di: Visualization, Investigation. XueQing Hu: Supervision, Validation. Yazhuo Wang: Visualization, Formal analysis. Ning Zhao: Supervision, Validation. XiaWei Zhang:Supervision, Validation. Yan Wang: Conceptualization, Supervision, Funding acquisition, Writing - review & editing. Ping Li: Supervision, Funding acquisition, Writing - review & editing. JingXia Zhao:Supervision,Writing - review & editing. All authors contributed to the article and approved the submitted version. Thanks to the above students and teachers who helped in the research and preparation of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2023.07.005.

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