

RESEARCH ARTICLE



Effects of Acupuncture on 1-chloro-2,4-dinitrochlorobenzene-induced Allergic Contact Dermatitis in Mice

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Abstract

Allergic contact dermatitis (ACD) is a chronic inflammatory skin disease. Topical corticosteroids are the first-line therapy for ACD despite their significant adverse effects. Acupuncture has been widely used in the treatment of various skin diseases, but its underlying mechanism remains unrevealed. In this study, we investigated the characteristics of acupuncture treatment based on effectiveness and mechanism. BALB/c mice received 1-chloro-2,4-dinitrobenzene (DNCB) application to build AD-like model. Results showed that acupuncture was an effective treatment method in inhibiting inflammatory conditions, serum IgE levels, and expression of proinflammatory cytokine Th2 (IL-4, IL-6), and Th2 (IL-1 β , TNF- α) mRNA compared with DNCB treatment. Acupuncture treatment also inhibited nuclear factor- κ B p65, phosphorylation of I κ B α , and phosphorylation of occludin proteins expression. Furthermore, it could improve the expression of epidermal growth factor in both mRNA and protein levels. These results suggest that acupuncture, as an alternative therapy treatment for its no significant side effects, was effective in alleviating ACD by reducing proinflammatory cytokines and changing proteins' expression.

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1. Introduction

Allergic contact dermatitis (ACD) is a paradigmatic, genetically complex disease involving gene–gene and gene–environment interactions. Both skin barrier defects and aberrant immune responses are believed to drive cutaneous inflammation in ACD. The prevalence of ACD has increased two to three folds during the last century, particularly in industrialized countries [1,2].

Corticosteroids, such as mometasone, have been the mainstay of therapy for ocular inflammatory diseases. Their temporary effective anti-inflammatory effects are often accompanied with numerous adverse effects including skin atrophy, characterized by a profound loss in skin thickness and elasticity combined with decreased barrier function [3,4]. Acupuncture is an ancient medical technic of China that can be traced back at least 2500 years [5]. It is now widely used as a complementary and alternative medicine in many countries. Acupuncture is becoming a popular way to modulate diverse immune disorders because it can be used for a long term with almost no side effects [6]. In several studies, acupuncture has been widely used in the treatment of pain, wounds, and various skin diseases, such as inflammation [7,8]. However, acupuncture's mechanism of action remains poorly understood.

Nuclear factor- κ B (NF- κ B) is a transcription factor that binds to promoters of many proinflammatory mediators and is considered to be a crucial regulator of inflammatory responses [9,10]. NF- κ B dimers are kept inactive in the cytoplasm through association with I κ B proteins, thus stimulating the activation of I κ B kinase complex, leading to phosphorylation, ubiquitination, and degradation of I κ B proteins. Released NF- κ B dimers translocate to the nucleus, binding specific DNA sequences, and promote transcription of target genes, whereas some proinflammatory cytokines drive the activation of NF- κ B in turn [11–14].

Epidermal growth factor (EGF) has biologically active polypeptides comprising 53 different amino acids which are involved in cell growth, differentiation, proliferation, metabolism, and skin regeneration [15,16]. EGF has been linked to ACD therapy, NF- κ B signaling, and the phosphorylation on tyrosine residues of occludin, which is required for its assembly into tight junctions.

Recent studies demonstrated that as an integral part, NF- κ B, EGF, and occludin are required for skin barrier balance [17]. In this study, therapeutic effects of acupuncture and mometasone were analyzed. This research revealed an advantage of acupuncture for skin barrier protection and faster skin regeneration using a 1-chloro-2,4-dinitrobenzene (DNCB)-induced mice model. This research also showed that acupuncture, combined with mometasone, could serve as the best effective therapy for ACD.

2. Materials and methods

2.1. Reagents

1-chloro-2,4-dinitrobenzene (DNCB) was purchased from Sigma-Aldrich (Sigma St. Louis, MO, USA). Other reagents

were purchased from Sinopharm Chemical Reagent Co. Ltd., China, unless otherwise specified.

2.2. Animals and treatment

Adult female BALB/c mice were purchased from the animal center of Guangzhou University of Chinese Medicine. Both animal care and the study protocol were conducted according to the guidelines of the Committee on Care and Use of Laboratory Animals of the animal center of Guangzhou University of Chinese Medicine. The mice were maintained for 7 days in pathogen-free conditions before the start of the experiment. Mice were kept at a constant temperature (23°C) and humidity (55%), with a 12-hour light/dark cycle, and they were provided with a laboratory diet and water *ad libitum*.

After the 10-day adaptation period, mice were randomly divided into six groups (each group, $n = 10$). Control group comprised mice without any stimulus and painted with phosphate buffer solution (PBS, pH7.4); DNCB group comprised mice sensitized with DNCB and painted with PBS; N-MP group comprised mice sensitized with DNCB and acupuncture at non-meridian point; MP group comprised mice sensitized with DNCB and acupuncture at meridian point [BL17 (geshu), BL20 (pi shu), and ST36 (zusanli)] for 10 minutes every other day (Fig. 1); MO group comprised mice sensitized with DNCB and painted with mometasone on each dorsal skin every other day; and MM group comprised mice sensitized with DNCB and painted with mometasone once after acupuncture every other day.

For the induction of ACD [18], the surfaces of both dorsal skins of mice were stripped with an electric razor (Nichiban, Japan). After stripping, 20 μ L of 1% DNCB (Sigma-Aldrich, St Louis, MO, USA) dissolved in acetone–olive oil solution (acetone: olive oil=1:3) was painted on each shaved dorsal skin once a day for 1 week (Days 1–7), and followed by a period of 0.5% DNCB applied repeatedly to the dorsal skin every other day for 3 weeks (Days 8–28) (Fig. 2). In the second challenge, the MP, MO, and MM groups were treated with acupuncture or mometasone 3 hours

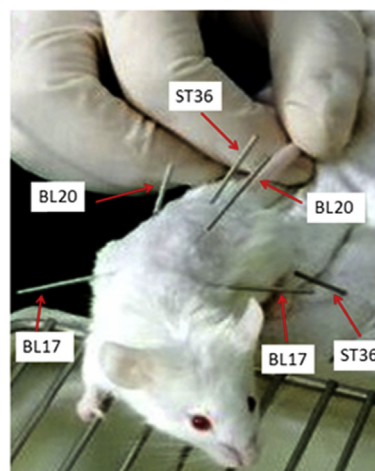


Figure 1 Body acupoints for acupuncture. Mice were treated at meridian point (geshu, pishu, zusanli) for 10 minutes every other day.

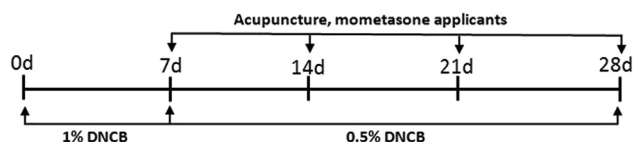


Figure 2 Study design for mice treatment. 1% 1-chloro-2,4-dinitrobenzene (DNCB) was painted on each shaved dorsal skin of mice once per day (d) for 1 week (Days 0–7), followed by a period of 0.5% DNCB applied repeatedly to the dorsal skin every other day for 3 weeks (Days 7–28). In the second challenge, the MP, MM, and MO groups were treated with acupuncture or mometasone 3 hours prior to the application of 0.5% DNCB (Days 7–28). MP group: mice sensitized with DNCB and treated at meridian points. MO group: mice sensitized with DNCB and painted with mometasone. MM group: mice sensitized with DNCB and painted with mometasone once after acupuncture every other day.

prior to the application of 0.5% DNCB (Days 8–28). Samples were collected and then fixed with 10% neutral buffered formalin solution or were frozen in liquid nitrogen for histopathologic examination, serum analysis, and expression pattern analysis on Day 28 of the experiment.

2.3. Measurement of scratch times and clinical score

For scratch times measurement, behaviors of mice were monitored according to the observation methodology of Kobayashi et al [19]. The frequency of scratching occurring on facial or dorsal skins was recorded with a 30-minute visual observation on the 1st day, 14th day, and 28th day after DNCB application.

For clinical score measurement, all mice were photographed to show the clinical symptoms on the 1st day, 14th day, and 28th day after DNCB application. Clinical symptoms of each mouse were evaluated, as previously described [11]. Briefly, erythema, edema, excoriation, and dryness on the dorsal surface were scored as 0 (not visible), 1 (mild), 2 (moderate), and 3 (severe), respectively. Scoring was performed by three independent observers.

2.4. Histological study

The fixed dorsal tissues were embedded in paraffin blocks; tissue sections (4–6 μ m) were mounted on slides, deparaffinized with xylene, rehydrated through graded alcohols, and stained with hematoxylin and eosin. Finally, the slides were observed using a Nikon Eclipse E600FN microscope (Nikon Instruments Inc., Melville, USA) at a fixed magnification of 200X.

2.5. Measurement of IgE levels in serum

Serum IgE levels were analyzed using the mouse IgG ELISA kit (BETHYL, Montgomery, TX, USA), as per the manufacturer's protocol. Antigen-specific IgE levels were indicated by optical density (OD). Mean absorbance of antigen-coated well minus mean absorbance of non-

coated well was used as the OD value of the mice-specific IgE levels.

2.6. Detection of mRNA expression by reverse transcription polymerase chain reaction

To determine gene expression in the dorsal skin, reverse transcription polymerase chain reaction (RT-PCR, real-time PCR) was performed. Total RNA was extracted from the dorsal skin using RNA extraction kit (Invitrogen, Los Angeles, CA, USA), according to manufacturer's instructions. The concentration of total RNA was quantified by measuring the absorbance at 260 nm. For cDNA synthesis using oligodT primers and Super-Script II reverse transcriptase (Invitrogen), 1.5 μ g of total RNA was used and subsequently diluted with nuclease-free water to 10 ng/ μ L cDNA. qPCR was performed utilizing hot-start SYBR green-based method (Invitrogen). Gene fold changes were determined by utilizing $2^{-\Delta\Delta C_t}$ method. DNA was amplified with an initial denaturation at 94°C for 4 minutes, followed by 40 cycles of 94°C (15 seconds) and 60°C (15 seconds). Primers are exhibited in Table 1. All experiments were performed in triplicate and repeated twice.

2.7. Detection of protein expression using Western blot

Western blotting was performed to study protein expression. Total protein was separated from each sample by electrophoresis on a 12% SDS-PAGE polyacrylamide gel and electrophoretically transferred onto polyvinylidene fluoride membranes (Bio-Rad Laboratories, Berkeley, California). The immunoblot was incubated overnight in blocking solution (5% skimmed milk) at 4°C followed by incubation with primary antibody. Then, the membranes were washed twice using 1x PBS and incubated with horseradish

Table 1 Primers used for quantitative real-time PCR analysis

Gene	Name	Primers (5' to 3')
IL-4	IL-4-F	CCCTTAAGAGGGATGCTGCC
	IL-4-R	AGGACGTTTGGCACATCCAT
IL-6	IL-6-F	GGCTAAGGACCAAGACCATCC
	IL-6-R	GCACTAGGTTTGCCGAGTAGA
IL-1 β	IL-1 β -F	TGCCACCTTTTGACAGTGATG
	IL-1 β -R	ATGTGCTGCTGCGAGATTTG
TNF α	TNF α -F	GATCGGTCCCAAGGGATG
	TNF α -R	GTGGTTTGTGAGTGTGAGGGT
EGF	EGF-F	CAGGAGGTCCGCTAGAGAAATG
	EGF-R	CAGAACAAATCCTGTGGGGC
GAPDH	GAPDH-F	CCCTTAAGAGGGATGCTGCC
	GAPDH-R	TACGGCCAAATCCGTTTACA

Primers were designed using Primer Express version 2.0 software. Primer specificity was confirmed using Primer-BLAST web software (National Centre for Biotechnology Information). EGF = epidermal growth factor; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; IL = interleukin; PCR = polymerase chain reaction; TNF = tumor necrosis factor.

peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, CA, USA) for 1–2 hours. The proteins were then visualized using an enhanced chemiluminescence detection reagent (Amersham Pharmacia, Piscataway, NJ, USA). The relative band density was determined using a computerized densitometry system and normalized to the β -actin signal from a blot developed under similar conditions.

2.8. Statistical analysis

The data are presented as the mean $\pm$ standard error and analyzed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Comparison among the groups was carried out by one-way analysis of variance. The comparison between two groups was carried out by least significant difference (LSD). A $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Effects of acupuncture on the symptoms of experimental ACD

First, it was determined if acupuncture treatment affected the symptoms of experimental ACD. Results showed that the ACD symptoms of MM, MP, and MO groups were significantly alleviated compared with DNCB and N-MP groups (Fig. 3A), including scratching frequency (Fig. 3B) and clinical scores (Table 2). Additionally, the scratching frequency of MM group was significantly lower than that of MO group. Interestingly, hyper pigmented patches and hardly any fur covering were seen in MO and MM groups, which involved treatment containing mometasone. In the MM group, acupuncture treatment could not ease the ACD symptoms, although there was a little improvement.

3.2. Effects of acupuncture on histopathological changes

To further explore the visual evaluation of ACD symptoms, histological analysis on the dorsal skins were performed by microscope. The epidermis and dermis in DNCB and N-MP groups showed strong edema and hyperplasia as well as massive infiltration of inflammatory cells (Fig. 4). The MO and MM groups had significantly reduced numbers of infiltrated immune cells and thickness of the epidermis compared with the DNCB and N-MP groups.

3.3. Effects of acupuncture on serum IgE levels

To further determine if suppression of ACD progression was associated with serum IgE levels, total serum IgE levels were measured. Compared with the control group, IgE levels were increased significantly in the DNCB and N-MP groups (Fig. 5). Furthermore, IgE levels of all the treatment groups were significantly decreased compared with the DNCB and N-MP groups. In addition, IgE levels of the MM group were lower than those of the MO group.

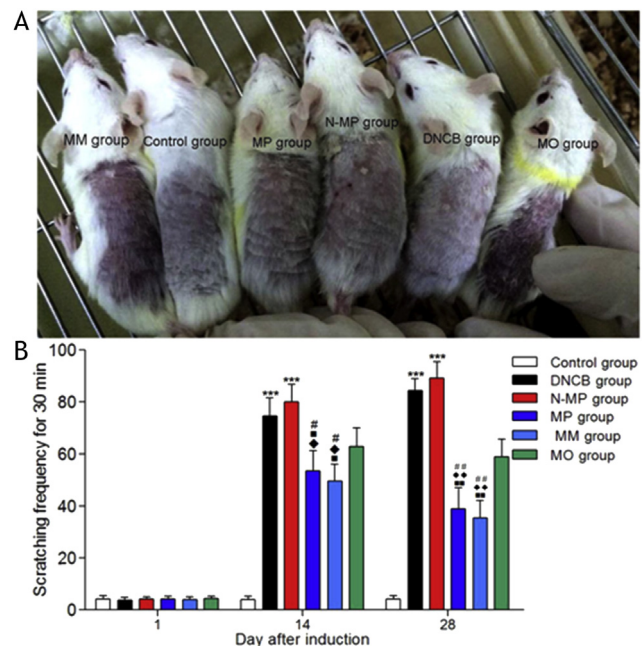


Figure 3 Effects of acupuncture on the symptoms of experimental allergic contact dermatitis. (A) The allergic contact dermatitis symptoms of different groups. (B) Scratching frequency of different groups. Data are the mean $\pm$ standard error of the mean ($n = 3$). Each bar represents the mean of three independent experiments carried out in triplicate. DNCB (1-chloro-2,4-dinitrobenzene) group: mice sensitized with DNCB. N-MP group: mice sensitized with DNCB and treated at non-meridian points. MP group: mice sensitized with DNCB and treated at meridian points. MM group: mice sensitized with DNCB and painted with mometasone once after acupuncture every other day. MO group: mice sensitized with DNCB and painted with mometasone. ■ $p < 0.05$ versus DNCB group; ◆ $p < 0.05$ versus N-MP group; # $p < 0.05$ versus MO group; *** $p < 0.0001$ versus control group; ■■ $p < 0.001$ versus DNCB group; ◆◆ $p < 0.001$ versus N-MP group; ## $p < 0.001$ versus MO group; min = minutes.

Table 2 Effects of acupuncture on the clinical score in different groups

Group	Day after induction		
	0 days	14 days	28 days
Control	0	0	0
DNCB	0	4.5 $\pm$ 0.31*	5.5 $\pm$ 0.42*
N-MP	0	4.7 $\pm$ 0.42*	5.8 $\pm$ 0.44*
MP	0	3.8 $\pm$ 0.38■◆#	3.5 $\pm$ 0.32■◆#
MM	0	3.7 $\pm$ 0.22■◆#	3.3 $\pm$ 0.31■◆#
MO	0	4.2 $\pm$ 0.31	4.1 $\pm$ 0.28

Note: Symbols used in this table match those used in several figures. Clinical scores of all the mice were recorded on the 1st day, 14th day, and 28th day after DNCB application. Scoring was performed by three independent observers. * $p < 0.05$ versus control group; ■ $p < 0.05$ versus DNCB group; ◆ $p < 0.05$ versus N-MP group; # $p < 0.05$ versus MO group. DNCB = 1-chloro-2,4-dinitrobenzene; MM = mometasone (immediately after acupuncture every other day); MO = mometasone (every other day); MP = meridian points; N-MP = non-meridian points.

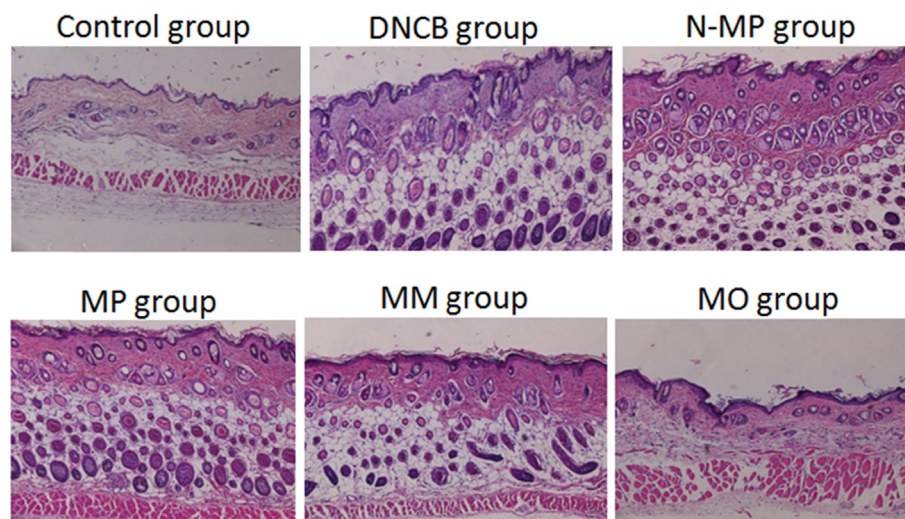


Figure 4 Effects of acupuncture on histopathologic changes by hematoxylin and eosin staining of epidermal and dermal hyperplasia. (A) Control group. (B) DNCB (1-chloro-2,4-dinitrobenzene) group. (C) N-MP group. (D) MP group. (E) MM group. (F) MO group. DNCB group: mice sensitized with DNCB. N-MP group: mice sensitized with DNCB and treated at non-meridian points. MP group: mice sensitized with DNCB and treated at meridian points. MM group: mice sensitized with DNCB and painted with mometasone at once after acupuncture every other day. MO group: mice sensitized with DNCB and painted with mometasone.

3.4. Effects of acupuncture on cytokine expression levels

The current authors wondered if proinflammatory cytokines were involved in suppression of ACD progression. Pathogenic cytokine expression levels were detected using an RT-PCR method. The data indicated that expression of interleukin (IL)-4, IL-6, tumor necrosis factor (TNF)- α , and IL-1 β mRNA was induced by DNCB treatment (Fig. 6). IL-4, TNF- α , and IL-1 β mRNA expressions were downregulated in the dorsal skin of the MP-, MM-, and MO-treated mice. In

addition, the above cytokine mRNA expression in the MM group was significantly lower than that of the MO group.

3.5. Effects of acupuncture on the I κ B α /NF- κ B pathway

To further elucidate the underlying mechanism of inhibitory effect of different treatments on inflammatory reaction, Western blot analysis was performed. The data demonstrated that all the treated groups showed suppression roles in the activation of NF- κ B signaling compared with the control group (Fig. 7). However, the expression of NF- κ B p65 and phosphorylation of I κ B- α were weaker in the MP, MM, and MO groups than in the DNCB and N-MP groups. Additionally, the MM group showed greater anti-inflammatory efficacy than the MO group. These data indicate that acupuncture plays an important role in the regulation of immune and inflammatory responses through inhibiting the NF- κ B signaling pathway.

3.6. Effects of acupuncture on EGF and occludin expression

To investigate the anti-inflammatory mechanism of acupuncture treatment in ACD-like disorders, the effects of acupuncture on regulation of EGF and occludin expression were examined by RT-PCR and Western blotting. The DNCB group could induce occludin expression (Fig. 8) and reduced EGF expression (Fig. 9) compared with the control group. On the contrary, acupuncture and mometasone enhanced EGF expression and decreased occludin expression compared with the DNCB group. Of the three treatment groups, the MM group was the most effective in improving expression of EGF (Fig. 9) as well as in inhibiting occludin.

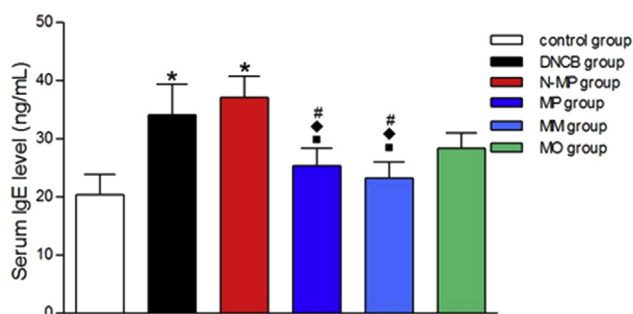


Figure 5 Effects of acupuncture on serum immunoglobulin (Ig)E levels. Data are the mean $\pm$ standard error of the mean ($n = 3$). Each bar represents the mean of three independent experiments carried out in triplicate. DNCB (1-chloro-2,4-dinitrobenzene) group: mice sensitized with DNCB. N-MP group: mice sensitized with DNCB and treated at non-meridian points. MP group: mice sensitized with DNCB and treated at meridian points. MM group: mice sensitized with DNCB and painted with mometasone at once after acupuncture every other day. MO group: mice sensitized with DNCB and painted with mometasone. * $p < 0.05$ versus control group; ■ $p < 0.05$ versus DNCB group; ◆ $p < 0.05$ versus N-MP group; # $p < 0.05$ versus MO group.

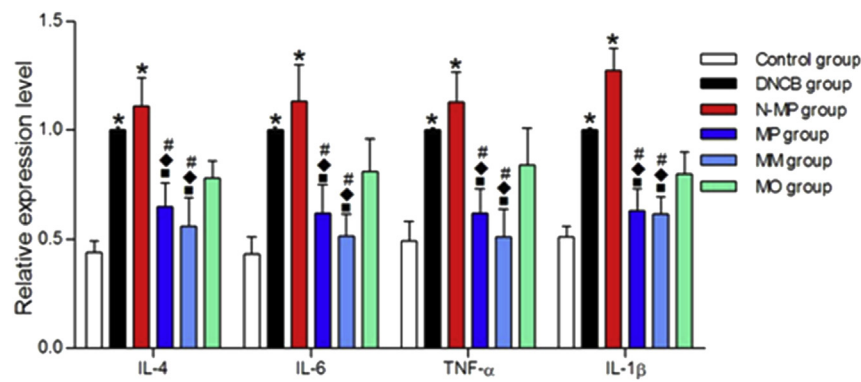


Figure 6 Effects of acupuncture on cytokine expression levels. Data are the mean $\pm$ standard error of the mean ($n = 3$). Each bar represents the mean of three independent experiments carried out in triplicate. DNCB (1-chloro-2,4-dinitrobenzene) group: mice sensitized with DNCB. NMP group: mice sensitized with DNCB and treated at non-meridian points. MP group: mice sensitized with DNCB and treated at meridian points. MM group: mice sensitized with DNCB and painted with mometasone at once after acupuncture every other day. MO group: mice sensitized with DNCB and painted with mometasone. * $p < 0.05$ versus control group; $\blacksquare p < 0.05$ versus DNCB group; $\blacklozenge p < 0.05$ versus NMP group; $\#p < 0.05$ versus MO group. IL = interleukin; TNF = tumor necrosis factor.

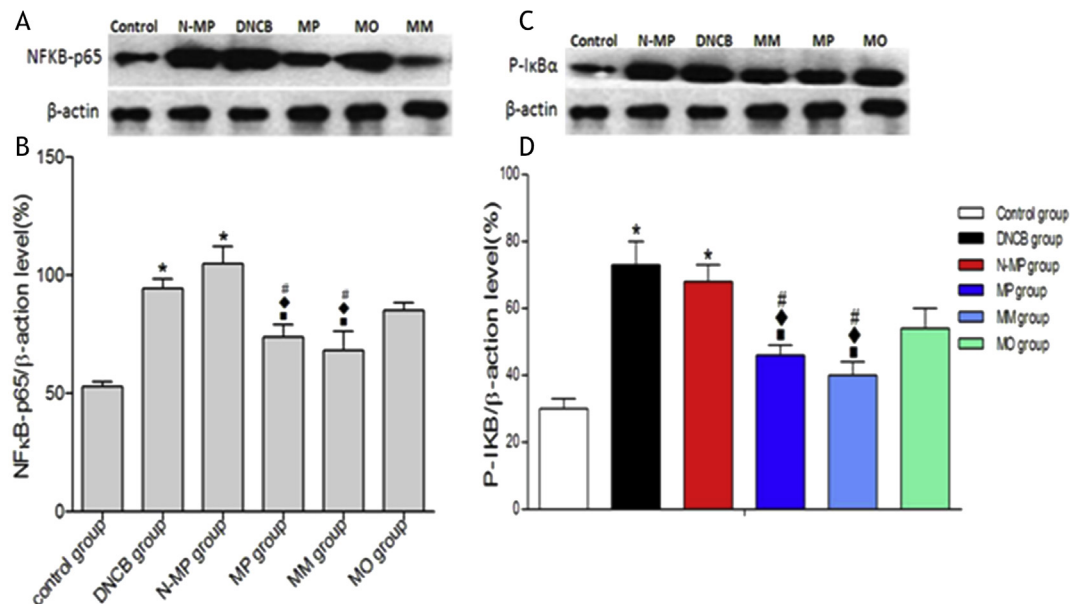


Figure 7 Effects of acupuncture on the $\text{I}\kappa\text{B}\alpha/\text{NF-}\kappa\text{B}$ pathway. (A and B) Western blots analysis of $\text{I}\kappa\text{B}\alpha/\text{NF-}\kappa\text{B}$ expression. (C and D) Western blots analysis of phosphorylated $\text{I}\kappa\text{B}\alpha$ expression. Data are the mean $\pm$ standard error of the mean ($n = 3$). Each bar represents the mean of three independent experiments carried out in triplicate. DNCB (1-chloro-2,4-dinitrobenzene) group: mice sensitized with DNCB. N-MP group: mice sensitized with DNCB and treated at non-meridian points. MP group: mice sensitized with DNCB and treated at meridian points. MM group: mice sensitized with DNCB and painted with mometasone at once after acupuncture every other day. MO group: mice sensitized with DNCB and painted with mometasone. * $p < 0.05$ versus control group; $\blacksquare p < 0.05$ versus DNCB group; $\blacklozenge p < 0.05$ versus N-MP group; $\#p < 0.05$ versus MO group.

4. Discussion

The skin is one of the most important and largest mammalian organs. It serves as a barrier providing protection from a wide variety of microbial, physical, and chemical insults. It is also considered as a major factor in the innate host defense system. ACD is an inflammatory, chronically relapsing, and intensely pruritic skin disease caused by complex pathogenic factors including skin barrier

dysfunction, bacterial infection, immune dysregulation, genetic susceptibility, and environment trigger.

IgE and cytokine expression have been known to cause both acute- and chronic-phase skin inflammations that are often associated with ACD [20]. Although generalized Th2-deviated immune response is closely linked to ACD, the skin disease itself is a biphasic inflammation with an initial Th2 phase while chronic lesions harbour Th0/Th1 cells [21]. Patients with ACD always have higher IgE levels and tend to

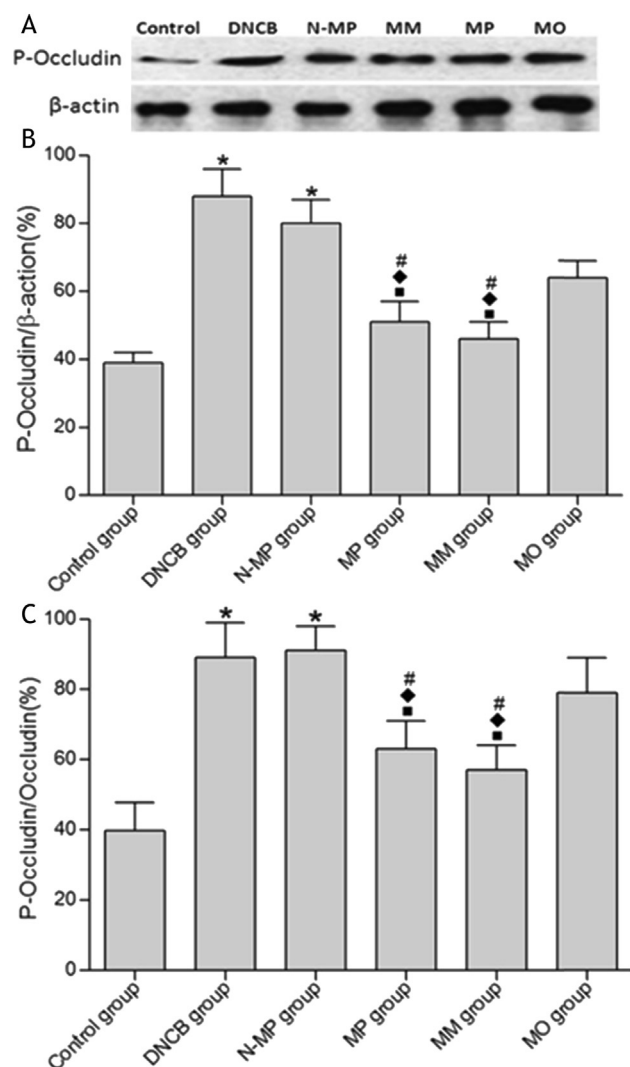


Figure 8 Effects of acupuncture on occludin expression. (A and B) Western blot analysis of phosphorylation of occludin (P-occludin) expression. (C) P-occludin and occluding ratio. Data are the mean $\pm$ standard error of the mean ($n = 3$). Each bar represents the mean of three independent experiments carried out in triplicate. DNCB (1-chloro-2,4-dinitrobenzene) group: mice sensitized with DNCB. N-MP group: mice sensitized with DNCB and treated at non-meridian points. MP group: mice sensitized with DNCB and treated at meridian points. MM group: mice sensitized with DNCB and painted with mometasone at once after acupuncture every other day. MO group: mice sensitized with DNCB and painted with mometasone. * $p < 0.05$ versus control group; ◆ $p < 0.05$ versus DNCB group; ■ $p < 0.05$ versus N-MP group; # $p < 0.05$, versus MO group.

secrete more IL-4 spontaneously. Previous studies have suggested that IL-4 and IL-13 impair expression and function of hBD2 and hBD3 in human epidermal keratinocytes, which might account for the increased susceptibility to skin infections seen in patients with ACD [22]. Likewise, in specimens with epidermal atrophy, intense IL-6 expression was detected. However, plasma was not elevated from patients with localized or systemic scleroderma, which suggests that IL-6 may be related to the pathophysiology of

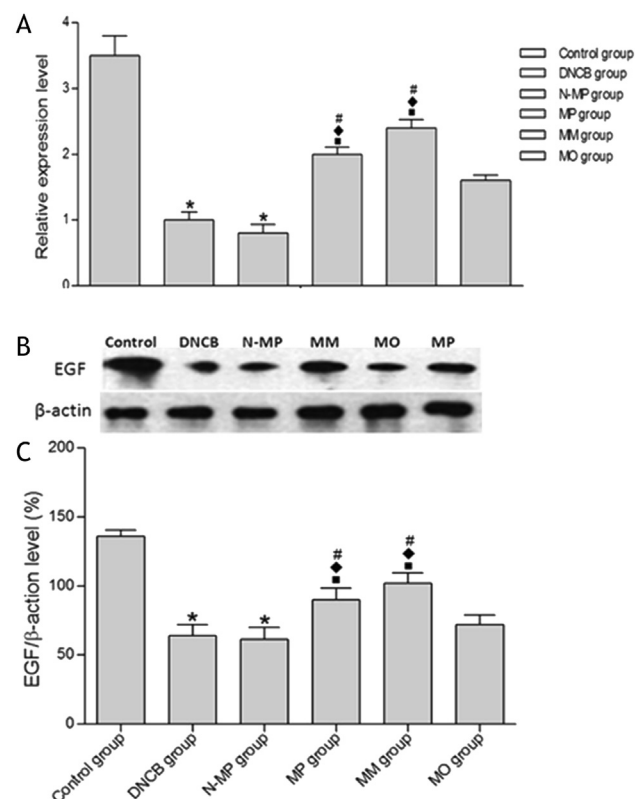


Figure 9 Effects of acupuncture on epidermal growth factor (EGF) expression. (A) DNA quantitative analysis of EGF expression (B and C) Western blots analysis of EGF expression. Data are the mean $\pm$ standard error of the mean ($n = 3$). Each bar represents the mean of three independent experiments carried out in triplicate. DNCB (1-chloro-2,4-dinitrobenzene) group: mice sensitized with DNCB. N-MP group: mice sensitized with DNCB and treated at non-meridian points. MP group: mice sensitized with DNCB and treated at meridian points. MM group: mice sensitized with DNCB and painted with mometasone at once after acupuncture every other day. MO group: mice sensitized with DNCB and painted with mometasone. * $p < 0.05$ versus control group; ◆ $p < 0.05$ versus DNCB group; ■ $p < 0.05$ versus N-MP group; # $p < 0.05$ versus MO group.

dermatologic diseases characterized by epidermal atrophy [23].

NF- κ B is a heterodimeric transcription factor of the Rel family that usually resides in the cytosol in an inactive form bound to the endogenous inhibitor of NF- κ B (I κ B) family proteins. I κ B kinase phosphorylates serine residues at the NH₂-terminus of I κ B during various inflammatory responses. The phosphorylated I κ B is immediately ubiquitinated and degraded in the 26S proteasome, leading to the release of NF- κ B and its translocation to the nucleus. The association of this released NF- κ B with its specific DNA binding sequences drives target genes and leads to cytokine production and cell proliferation. Growing evidences had demonstrated the role of NF- κ B signaling in the immunological disturbance that was observed in ACD. Martin et al [24] reported that topical application with a new NF- κ B inhibitor improves ACD in NC/NgaTnd mice. Christopher et al [25] explored the possibility of using topical NF- κ B

decoy as a novel therapeutic alternative for targeting Th1/Th2-driven skin inflammation in experimental ACD.

As an essential part of skin regeneration growth factor, EGF supports cell renewal by assisting in the synthesis of proteins, increasing circulation, metabolism, mitosis, cell growth, differentiation, and blood-vessel formation [26]. It was reported that chronic skin damage induced by DNCB as well as their frequent scratch displayed a downregulation expression of EGF and its mRNA in mice [15]. Downregulation of EGF and mislocalization of EGF receptor in the cytoplasm of keratinocytes probably contribute to an inhibition of epithelialization in chronic skin damage. Conversely, it appears to differ in acute skin damage. Present study showed that the healing process for ACD is satisfactory in the PPI group in which a severe EGF immunohistochemical reaction could be observed [27]. Moreover, a previous non-clinical study concluded that the EGF vaccination in mice decreased the normal croton oil-induced inflammation response without apparent impairment in tissue healing [28]. EGF-dependent signaling pathways that facilitate cell growth and re-epithelization via binding to the EGF receptor localized through the entire epidermis [29].

Tight junctions (TJs) form a selective barrier to the diffusion of toxins, allergens, and pathogens from the external environment. As an important component of TJs, occludin plays an important role in the regulation of epithelial TJs [30]. Tyrosine phosphorylation of occludin on specific residues results in loss of its interaction with ZO-1 and therefore disassembly of TJs [31]. Furthermore, a recent *in vitro* study demonstrated that Tyr phosphorylation of the C-terminal region of occludin reduces its ability to interact with ZO-1 [32,33]. Hydrogen peroxide-induced barrier dysfunction was attenuated by pretreatment of cell monolayers with EGF [34]. Protein kinase C (PKC)-mediated protection of TJs by EGF was also demonstrated in Mz-Ch1 cell monolayers, a human cholangiocyte cell line [35].

In this study, we aimed to investigate the roles of IgE, cytokine, NF- κ B, EGF, and occludin in ACD using DNCB-induced model in mice. The results revealed that compared with control group, serum IgE level and cytokine expression were increased significantly in the DNCB group. In addition, I κ B α /NF- κ B pathway was inhibited in the DNCB-induced group including higher expression of NF- κ B-p65 and phosphorylation of I κ B α proteins than the control group. Furthermore, we also detected increased occludin expression in DNCB-induced model through Western blotting analysis. The current data indicated that the dysregulated proteins might play a central role in the progression and maintenance of ACD. Therefore, therapies focusing on changing expression of the above proteins might be beneficial for patients with ACD.

As the first-line therapy for ACD, the beneficial therapeutic effects of topical corticosteroids (mometasone) are often accompanied by numerous adverse effects including skin atrophy, characterized by a profound loss in skin thickness and elasticity combined with decreased barrier function. Acupuncture is an ancient medical technic of China that can be traced back at least 2,500 years. It is now widely used as a complementary and alternative medicine in many countries [36,37]. The therapeutic efficacy of acupuncture, with hardly any adverse effects, for treating ACD has been proven in many studies especially with

respect to reducing experimentally-induced itching, allergen-induced basophil activation, and eczema in ACD.

Although acupuncture treatment has been increasingly used for ACD, with several clinical studies demonstrating the effectiveness of acupuncture [38], preclinical studies, especially *in vivo* studies, investigating the mechanism of acupuncture for treating ACD are lacking.

In this study, therapeutic effects of acupuncture and mometasone were compared using a DNCB-induced mice model. First, the ACD symptoms of MM and MP groups were alleviated significantly compared with the DNCB and MO groups; these symptoms included scratching frequency and clinical scores. Second, the MM and MO groups had significantly reduced numbers of infiltrated immune cells and thickness of epidermis compared with DNCB and MP groups. Third, serum IgE levels of MM and MO groups were decreased significantly compared with DNCB and MO groups. These results indicate that acupuncture treatment could markedly reduce symptoms induced by DNCB and is a relatively effective method for treating ACD.

Then, an analysis was conducted to determine the effects of acupuncture on cytokine, NF- κ B, EGF, and occludin expression that were key factors for the progression for ACD. Western blot analysis revealed that cytokine, NF- κ B-p65, and phosphorylation of I κ B α expression were significantly increased in MM and MP groups compared with DNCB and MO groups. In addition, occludin expression was also increased in MM and MP groups. However, the MM and MP groups had EGF expression compared with the DNCB and MO groups. These results demonstrated that acupuncture was an effective therapy for ACD.

There were some novel mentions in our study compared with the study by Park et al [39]. First, we chose ACD as a research model; however, their research field involved atopic dermatitis. Furthermore, we investigated the effects of acupuncture on the regulation of EGF and occludin expression by RT-PCR and Western blotting, which was not involved in their study.

In conclusion, these experimental results suggest that acupuncture treatment had anti-inflammatory effects that were produced by inhibiting proinflammatory activities. Acupuncture probably also accelerated skin regeneration and skin barrier protection by promoting EGF secretion and inhibiting Tyr phosphorylation of occludin. Long-term use of topical corticosteroids for skin inflammation poses risks of systemic and local side effects. Therefore, the current study's results suggest that acupuncture combined with mometasone might be a better choice for ACD treatment, at least in mice. However, cautious and deeper research is indispensable to assess the effect of acupuncture on ACD treatment in human beings.

Disclosure statement

The authors affirm that they have no conflicts of interest or no financial interests related to the material of this manuscript.

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