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Research article

p-coumaric acid, an active ingredient of *Panax ginseng*, ameliolates atopic dermatitis-like skin lesions through inhibition of thymic stromal lymphopoietin in mice

Phil-Dong Moon [1,☆], Na-Ra Han [2,☆], Jin Soo Lee [2], Hyung-Min Kim [2,*], Hyun-Ja Jeong [3,**]

[1] *Center for Converging Humanities, Kyung Hee University, Seoul, Republic of Korea*
[2] *Department of Pharmacology, College of Korean Medicine, Kyung Hee University, Seoul, Republic of Korea*
[3] *Division of Food and Pharmaceutical Engineering, BioChip Research Center, Hoseo University, Asan, Chungnam, Republic of Korea*

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ABSTRACT

Background: Atopic dermatitis (AD) is associated with chronic skin inflammatory reactions. *p*-coumaric acid (*p*CA) is an active ingredient of *Panax ginseng* Meyer (Araliaceae).
Methods: Here, we estimated an anti-AD effect of *p*CA on activated mast cells, activated splenocytes, and a mouse model of AD. Cytokines levels were measured by ELISA and protein activation was analyzed by Western blotting. 2,4-dinitrofluorobenzene (DNFB) was used to induce AD-like skin lesions.
Results: The treatment with *p*CA suppressed the productions and mRNA expressions of thymic stromal lymphopoietin (TSLP), TNF-α, IL-6, and IL-1β in HMC-1 cells. *p*CA downregulated the expressions of RIP2 and caspase-1, phosphorylated-(p)p38/pJNK/pERK, and pIKKβ/pIkBα/NF-κB in HMC-1 cells. *p*CA also decreased the productions of TSLP, TNF-α, IL-6, IL-4, and IFN-γ in the supernatant of stimulated splenic cells. Comparing to DNFB-sensitized control group, *p*CA-treated group alleviated pathological changes of AD-like lesions. *p*CA decreased the proteins and mRNA expressions levels of TSLP, IL-6, and IL-4 in the skin lesions. Caspase-1 activation was also downregulated by *p*CA treatment in the AD-like lesions. The serum levels of histamine, IgE, TSLP, TNF-α, IL-6, and IL-4 were suppressed following treatment with *p*CA.
Conclusion: This study suggests that *p*CA has the potential to improve AD by suppressing TSLP as well as inflammatory cytokines via blocking of caspase-1/NF-κB signal cascade.
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1. Introduction

Atopic dermatitis (AD) is a recurrent and chronic inflammatory skin disorder characterized by debilitating itch, signs of anxiety and depression, impaired quality of life, sleep deprivation, and decreased productivity [1]. AD has become a worldwide public health problem with increasing prevalence in most countries [2].

In atopic disorders including AD and asthma, cytokine thymic stromal lymphopoietin (TSLP) has a fundamental role. A severe AD development was resulted from overexpressed TSLP in the skin of mice [3]. On the contrary, impairment of AD-like skin lesions was resulted from knockout of TSLP in the skin of mice [4]. Epithelial cells, keratinocytes as well as mast cells are closely involved in atopic diseases [5]. Mast cell activation was elevated in atopic disorder models, suggesting a role of mast cells in atopic disorder [6–8].

Caspases are involved in apoptosis, whereas caspase-1 which regulates inflammatory responses, is an inflammatory caspase and [9,10]. Deletion of caspase-1 protects mice from chemical-induced intestinal inflammation [11]. The caspase-1 inhibitor down-regulated NF-κB activation, presenting that caspase-1 acts the up-stream of NF-κB [12]. In a preliminary study, TSLP expression was regulated by caspase-1 and NF-κB signaling in HMC-1 cells [12].

Coumaric acid is a hydroxycinnamic acid compound that naturally occurs as three types of *m*-, *o*-, and *p*-coumaric acid (*p*CA, supplementary fig. 1A). Among them, *p*CA is the most commonly appearing isomer in nature [13]. *p*CA is an active ingredient of fruit, leaves, and roots of *Panax ginseng* Meyer (Araliaceae) [14,15].

* Corresponding author. Department of Pharmacology, College of Korean Medicine, Kyung Hee University, Seoul, 02447, Republic of Korea
** Corresponding author. Division of Food and Pharmaceutical Engineering, BioChip Research Center, Hoseo University, Asan, Chungnam, 31499, Republic of Korea
E-mail addresses: hmkim@khu.ac.kr (H.-M. Kim), hjjeong@hoseo.edu (H.-J. Jeong).
☆ P.-D. Moon and N.-R. Han contributed equally to this work.

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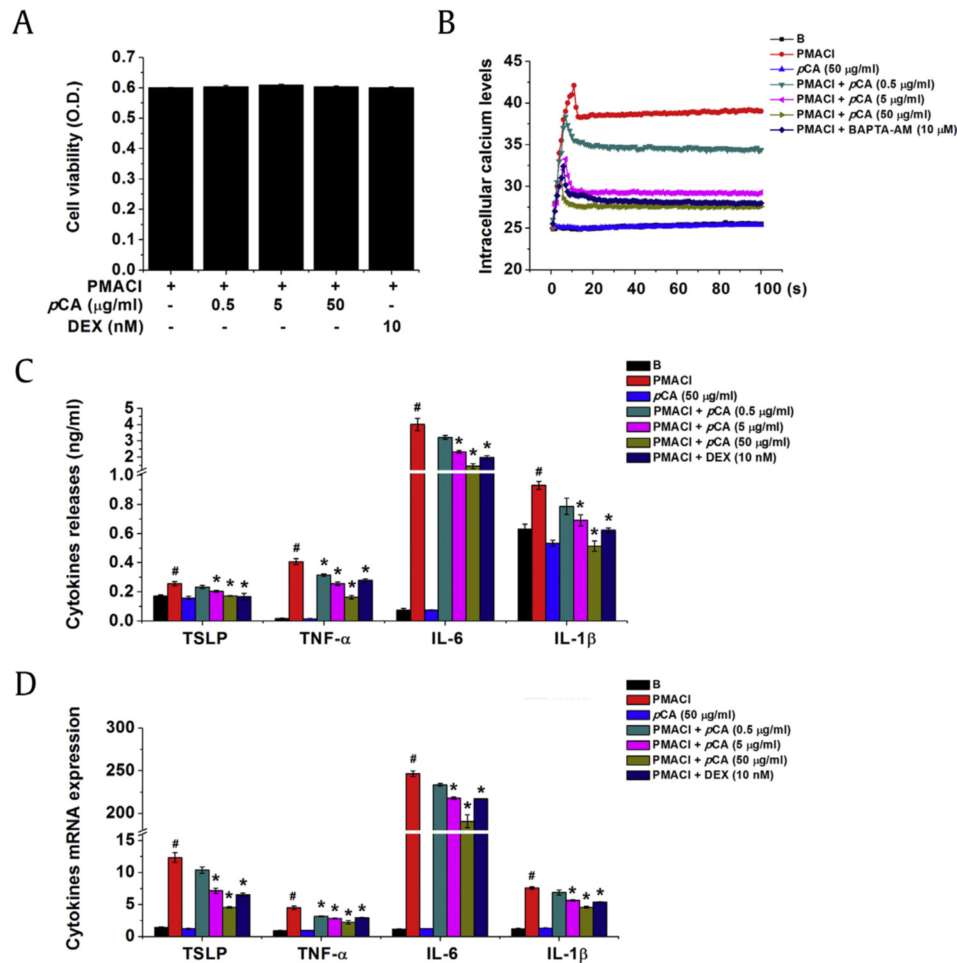

Fig. 1. Effects of *p*CA on inflammatory cytokines levels in activated HMC-1 cells. (A) *p*CA or DEX was added in HMC-1 cells 1 h before PMACI, and then the cells were incubated for 7 h. Cytotoxicity was assessed using an MTT. (B) *p*CA or BAPTA-AM was added in fura-2/AM-pretreated HMC-1 cells 20 min before PMACI. B, Blank (inactivated cells); PMACI, PMACI-activated cells. (C) *p*CA or DEX was added in HMC-1 cells (4×10^5) 1 h before PMACI, and then the cells were incubated for 7 h. The productions were assessed using ELISA. (D) *p*CA or DEX was added in HMC-1 cells (1×10^6) 1 h before PMACI, and then the cells were incubated for 5 h. The mRNA expressions were assessed by means of real time-PCR. The levels of each mRNA were normalized to the levels of GAPDH. $^{\#}p < 0.05$ compared with inactivated group. $^{*}p < 0.05$ compared with PMACI-activated group.

P. ginseng exerts various activities such as anticancer, antioxidant, or antiinflammatory properties [15,16]. *p*CA exerts diverse beneficial impacts including anti-neoplastic, anti-Alzheimer, anti-oxidant, anti-inflammatory, neuroprotective, anti-mutagen, anti-microbial, and UV-protection [17]. *P. ginseng* and ginsenosides have known to be effective in treating various skin diseases, including AD [18]. However, the regulatory mechanisms of *p*CA in AD have not been fully described. Thus, we explored whether *p*CA can ameliorate AD by means of *in vitro* and *in vivo* models.

2. Materials and methods

2.1. pCA and dexamethasone (DEX)

*p*CA (purity ≥ 98%, Fluka™, Mexico City, Mexico) was prepared according to studies of Kim et al [19] and Cha et al [13]. DEX was solved considering study of Chen et al [20].

2.2. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

An MTT (Sigma Chemical Co.) assay was performed to estimate cytotoxicity, as described previously [21].

2.3. Intracellular calcium assessment

The intracellular calcium levels were examined using a fluorospectrometer (excitation 360nm, emission 450nm, Thermo Fisher Scientific, Waltham, MS, USA) for 100 s, as previously described [22].

2.4. Measurement of cytokines and IgE levels

The values of TSLP, TNF-α, IL-6, IL-1β, IL-4, IFN-γ, and IgE were measured by means of ELISA method (Pharmingen, Sandiego, CA, USA; R & D system Inc., Minneapolis, MN, USA), as previously described [23].

2.5. Quantification of gene expression

Polymerase chain reaction (PCR) was conducted considering the study of Han et al [24].

2.6. Western bloting

Western blotting was conducted considering the study of Moon et al [25].

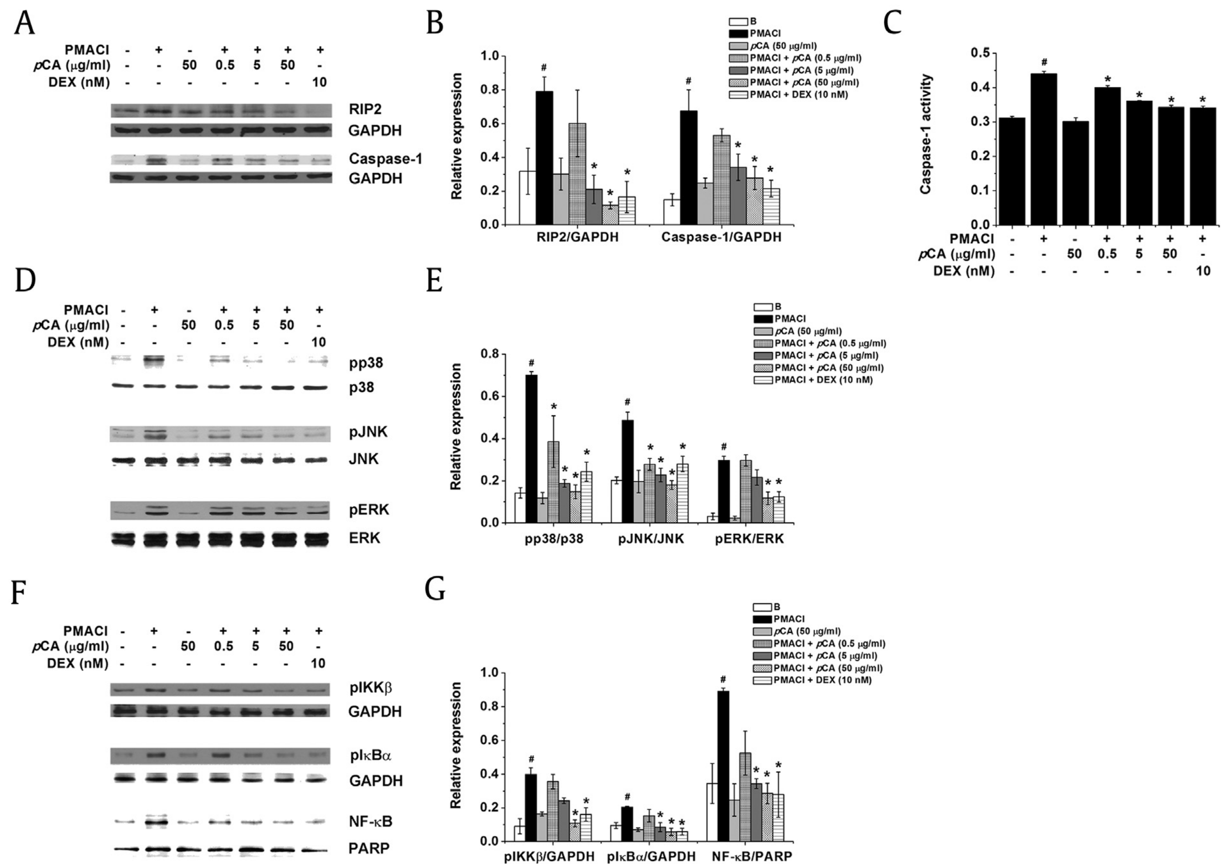

Fig. 2. Effects of *p*CA on RIP2/caspase-1, MAPKs, and pIKKβ/pIκBα/NF-κB expressions in activated HMC-1 cells. (A) *p*CA or DEX was added in HMC-1 cells (5×10^6) 1 h before PMACI, and then the cells were incubated for 30 min. RIP2 and caspase-1 expressions were analyzed using Western blot. (B) The each intensity was quantified by densitometry and the relative expressions are presented as RIP2/GAPDH and caspase-1/GAPDH. (C) The caspase-1 activity was assessed by means of a caspase-1 assay kit. (D) *p*CA or DEX was added in HMC-1 cells (5×10^6) 1 h before PMACI, and then the cells were incubated for 30 min. MAPKs expressions were analyzed with Western blotting. (E) The each intensity was quantified by densitometry and the relative expressions are presented as pp38 to p38, pJNK to JNK, and pERK to ERK. (F) *p*CA or DEX was added in HMC-1 cells (5×10^6) 1 h before PMACI, and then the cells were incubated for 1 h (pIKKβ) or 2 h (pIκBα, and NF-κB). The expressions of pIKKβ in total proteins, pIκBα in cytosolic extract, and NF-κB in nuclear extract were analyzed using Western blotting. (G) The relative intensities were quantified based on the intensities of GAPGH or PARP bands. B, Blank (inactivated cells); PMACI, PMACI-activated cells. $^{\#}p < 0.05$ compared with inactivated group. $^{*}p < 0.05$ compared with PMACI-activated group.

2.7. Caspase-1 activity assay

The caspase-1 activities in the lysates and lesions homogenates were determined by means of a kit from R & D system, as described previously [26].

2.8. Nuclear and cytoplasmic extract preparation

Harvested HMC-1 cells were used to prepare cytoplasmic and nuclear extracts, as previously described [21].

2.9. AD-like skin lesions induction

All treatment and procedure complied with internationally accepted principles for laboratory animal care according to the United States guidelines (NIH publication no. 85-23, revised in 1985). General treatment and process of an AD mouse model are shown in supplementary fig. 1B. AD-like skin lesions were induced, as described previously [24]. Ethics for animal study (KHUASP (SE)-18-022) was approved by the animal care committee of Kyung Hee University.

2.10. Histological analysis

The mast cells, inflammatory cells, and epidermal thickness in de-paraffinized and re-hydrated tissue slides were visualized by means of hematoxylin and eosin (H&E) or toluidine blue staining, as previously described [4].

2.11. Histamine assay

Serum histamine was evaluated by *o*-phthaldialdehyde (Sigma Chemical Co.) fluorospectrometric procedure, as described previously [27].

2.12. Statistical analysis

Each value was showed as mean ± standard error of mean (SEM). A SPSS statistical program (version 25, IBM) was used. Independent *t*-test was used to compare the medians of two groups {B vs (PMA plus A23187; PMACI), vehicle vs control}. ANOVA with Tukey post hoc test was employed to compare differences among PMACI/control group vs *p*CA or DEX-treated groups. *P* values lower than 0.05 were considered to be significant.

Table 1
Effects of *p*CA on the Productions of inflammatory cytokines from activated splenocytes

	B	-	*p*CA (50 μg/ml)	*p*CA (0.5 μg/ml)	*p*CA (5 μg/ml)	*p*CA (50 μg/ml)	DEX (10nM)
	-	CD3/CD28	-	CD3/CD28	CD3/CD28	CD3/CD28	CD3/CD28
TSLP (ng/ml)	0.19 ± 0.02	0.56 ± 0.11[#]	0.16 ± 0.02	0.54 ± 0.02	0.41 ± 0.03	0.16 ± 0.02*	0.33 ± 0.01*
TNF-α (ng/ml)	0.11 ± 0.02	0.47 ± 0.07[#]	0.11 ± 0.05	0.45 ± 0.01	0.39 ± 0.01	0.29 ± 0.01*	0.27 ± 0.02*
IL-6 (ng/ml)	0.03 ± 0.00	1.00 ± 0.19[#]	0.02 ± 0.00	1.05 ± 0.13	0.70 ± 0.06	0.42 ± 0.01*	0.24 ± 0.01*
IL-4 (ng/ml)	0.00 ± 0.00	0.40 ± 0.10[#]	0.00 ± 0.00	0.32 ± 0.01	0.22 ± 0.02	0.13 ± 0.00*	0.05 ± 0.00*
IFN-γ (ng/ml)	0.00 ± 0.00	29.96 ± 5.38[#]	0.00 ± 0.00	11.72 ± 1.06*	5.97 ± 1.40*	1.28 ± 0.01*	1.62 ± 0.22*

Splenocytes (2.5 × 106/ml) were stimulated with immobilized anti-CD3/soluble anti-CD28 antibodies and treated with *p*CA or DEX for 24 h. The productions of inflammatory cytokines were measured by using ELISA. B, Blank (unstimulated cells); CD3/CD28, anti-CD3/anti-CD28 antibodies-stimulated cells. #$p < 0.05$ compared with unstimulated group. *$p < 0.05$ compared with anti-CD3/CD28 antibodies-stimulated group.

3. Results

3.1. *p*CA treatment alleviated inflammatory cytokines levels in HMC-1 cells

In general, inflammatory cytokines released from mast cells play a critical role during induction of AD [28]. Thus, we investigated a regulatory effect of *p*CA on inflammatory cytokines levels in activated HMC-1 cells. First, we found that 0.5, 5, and 50 μg/ml of *p*CA and 10mM of DEX did not show cytotoxicities in activated HMC-1 cells (Fig. 1A). The protein kinase C activator plus calcium ionophore (PMACI)-stimulation showed a marked increase in intracellular calcium level. However, treatment with *p*CA led to a significant downregulation in intracellular calcium levels (Fig. 1B). The therapeutic potential on intracellular calcium level in *p*CA (50 μg/ml)-treated group was comparable with calcium chelator BAPTA-AM-treated group in HMC-1 cells. The treatment with *p*CA (5 and 50 μg/ml), alike treatment with DEX, reduced the productions and mRNA expressions of TSLP, IL-6, IL-1β, and tumor necrosis factor (TNF)-α, in HMC-1 cells ($p < 0.05$; Fig. 1C and D). *p*CA alone did not produce a significant change on these levels (Fig. 1).

3.2. *p*CA treatment reduced RIP2 and caspase-1 expressions in activated HMC-1 cells

For investigation about the inhibitory mechanism of *p*CA on inflammatory cytokines, we measured the expression levels of receptor interacting protein 2 (RIP2) and caspase-1 in activated HMC-1 cells. As indicated in Fig. 2A and B, Western blotting showed that PMACI addition significantly upregulated the activation levels of RIP2 and caspase-1 ($p < 0.05$). 5 and 50 μg/ml of *p*CA treatment significantly suppressed the activation levels of RIP2 and caspase-1 in activated HMC-1 cells ($p < 0.05$; Fig. 2A and B). In addition, *p*CA (0.5, 5, and 50 μg/ml) decreased the activities of caspase-1 in activated HMC-1 cells ($p < 0.05$; Fig. 2C). *p*CA itself did not influence in inactivated HMC-1 cells (Fig. 2A–C). The alleviated expressions of RIP2 and caspase-1 in *p*CA (50 μg/ml)-treated group were comparable with those of DEX-treated group in HMC-1 cells (Fig. 2A–C).

3.3. *p*CA treatment attenuated MAPKs and pIKKβ/pIkBα/NF-κB expressions in activated HMC-1 cells

We further evaluated whether *p*CA would down-regulate MAPKs, p38/c-Jun N-terminal kinase (JNK)/extracellular signal-regulated kinase (ERK) and pIKKβ/pIkBα/NF-κB signaling pathways which play a pivotal role in inflammatory diseases [29]. As shown in Fig. 2D and E, PMACI addition significantly upregulated the phosphorylation levels of p38, JNK, and ERK compared to those of inactivated group, B ($p < 0.05$). The treatment with *p*CA (50 μg/ml), alike DEX-treated group, significantly decreased the expression levels increased by PMACI ($p < 0.05$; Fig. 2D and E). Also, PMACI addition upregulated the phosphorylated-(p)IKKβ, pIkBα, and NF-κB expression levels ($p < 0.05$; Fig. 2F and G). *p*CA (50 μg/ml), alike DEX-treated group, reduced the expression levels ($p < 0.05$; Fig. 2F and G). *p*CA alone did not produce a significant change on these levels (Fig. 2D–G).

3.4. *p*CA treatment decreased inflammatory cytokines in activated splenocytes

Activation with anti-CD3 and anti-CD28 antibodies results in the activation of primary splenic cells and the production of inflammatory cytokines [30,31]. Hence, to identify an inhibitory effect of *p*CA treatment on splenic T cells-derived inflammatory cytokines, we estimated TSLP, TNF-α, IL-6, IL-4, and IFN-γ levels on supernatant in anti-CD3 and anti-CD28 antibodies-stimulated splenic cells. Table 1 showed that the productions of TSLP, TNF-α, IL-6, IL-4, and IFN-γ upregulated by anti-CD3 and anti-CD28 antibodies were inhibited by 50 μg/ml of *p*CA ($p < 0.05$). These inhibitory effects of *p*CA were comparable to DEX group.

3.5. *p*CA treatment alleviated AD-like symptoms from skin lesions

We figured out that *p*CA treatment ameliorated the levels of inflammatory cytokines *in vitro*. Hence, we tried to verify whether *p*CA regulates pathological symptoms of AD-like skin lesions in a 2,4-dinitrofluorobenzene (DNFB)-induced AD mouse model. Fig. 3A showed that *p*CA treatment markedly alleviated the striking hemorrhage, excoriation, and erosion compared to control group in DNFB-induced AD-like skin lesions. *p*CA treatment reduced the epidermal thickening as well as accumulation of inflammatory cells (middle panel, $p < 0.05$) and mast cells (lower panel, $p < 0.05$) in the lesions (Fig. 3B and C). In addition, *p*CA treatment significantly inhibited the scratching behavior ($p < 0.05$; Fig. 3D). Treatment with DEX, as observed in the *p*CA-treated group, alleviated the pathological symptoms of AD-like skin lesions (Fig. 3).

3.6. *p*CA treatment inhibited inflammatory cytokine levels and caspase-1 activation in the skin lesions

Next, we investigated whether *p*CA can reduce TSLP, IL-6, and IL-4 levels in the skin lesions. TSLP, IL-6, and IL-4 protein levels were suppressed by *p*CA ($p < 0.05$; Fig. 4A). Also, *p*CA suppressed the TSLP, IL-6, and IL-4 mRNA expressions in the skin lesions ($p < 0.05$; Fig. 4B and C). DEX decreased the TSLP, IL-6, and IL-4 proteins and mRNA expressions ($p < 0.05$; Fig. 4A–C). Treatment with *p*CA reduced inflammatory cytokines levels through blocking of caspase-1 signal cascade in HMC-1 cells. Hence, we finally investigated whether *p*CA can modulate protein expression and activity of caspase-1 in the skin lesions. DNFB sensitization enhanced the protein levels of caspase-1, whereas *p*CA or DEX significantly

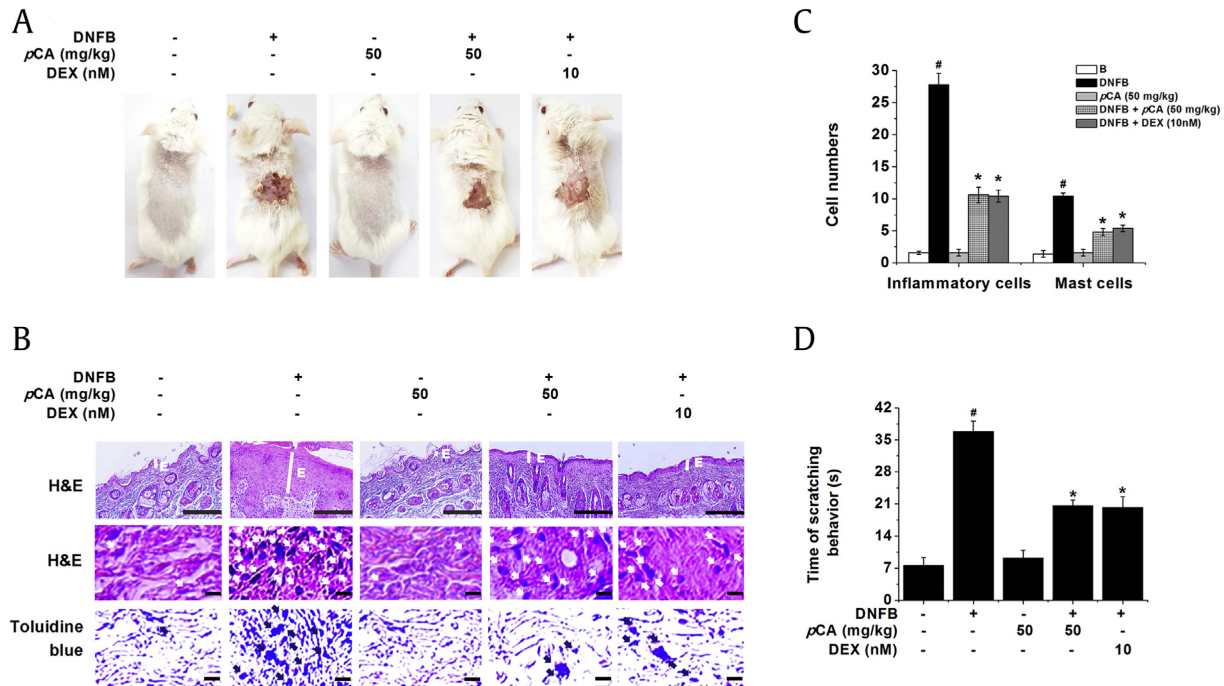

Fig. 3. Effects of *p*CA on the clinical symptoms in DNFB-induced AD-like mouse model. (A) Representative clinical characteristics was observed after the final DNFB sensitization. (B) H&E sections of skin lesions show epidermal thickness (upper panel, scale bar = 150 μm) and inflammatory cells (middle panel, scale bar = 9 μm) in the lesions. E, epidermis. Toluidine blue sections show mast cells in the lesions (lower panel, scale bar = 9 μm). Arrows indicate inflammatory cells and mast cells. (C) Inflammatory cells and mast cells were counted. $n = 5$ sections per data point. (D) Scratching time was counted for 10 min. $^{\#}p < 0.05$ compared with vehicle group. $^{*}p < 0.05$ compared with DNFB control group. $n = 5$.

reduced the protein expressions of caspase-1 in the skin lesions ($p < 0.05$; Fig. 4D and E). *p*CA or DEX significantly alleviated activities of caspase-1 in the skin lesions ($p < 0.05$; Fig. 4F).

3.7. *pCA treatment decreased the serum histamine, IgE, and inflammatory cytokines in AD-like mouse model*

Histamine and IgE induces inflammatory cell infiltration and scratching behaviors, and elevates inflammatory cytokines levels in AD [32]. Thus, we estimated the histamine, IgE, TSLP, IL-6, IL-4, and TNF-α, levels in the serum of DNFB-sensitized mice. Table 2 showed that *p*CA or DEX reduced the histamine, IgE, TSLP, IL-6, IL-4, and TNF-α levels increased by DNFB-sensitization ($p < 0.05$).

4. Discussion

In general, protein kinase C (PKC) activation and intracellular calcium upregulation result from mast cell activation by binding with antigens [33]. To reenact this circumstance, we used PMA for PKC activation and calcium ionophore for intracellular calcium

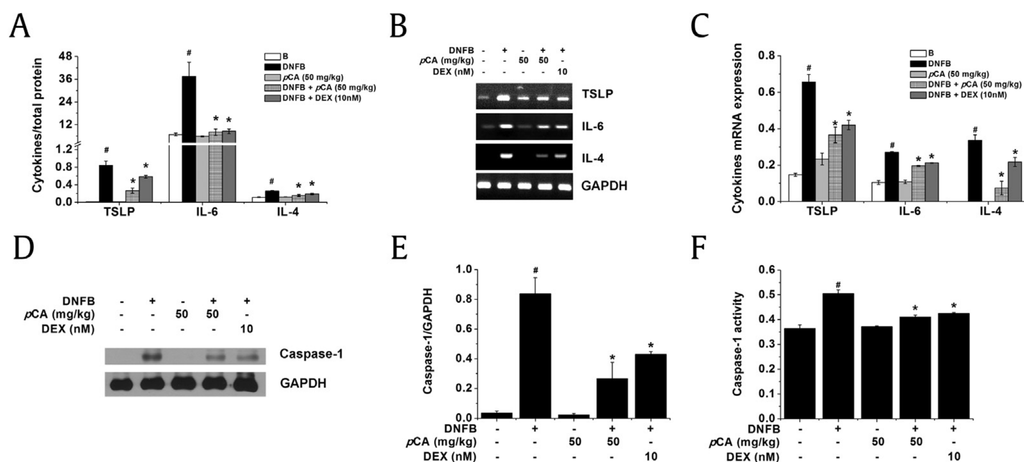

Fig. 4. Effects of *p*CA on inflammatory cytokines levels and caspase-1 activation in the skin lesions of DNFB-induced AD-like mouse model. (A) The protein expressions of TSLP, IL-6, and IL-4 in the skin lesion homogenate were measured by using ELISA. A bicinchoninic acid was used to assess total protein concentrations. (B) The mRNA expressions of TSLP, IL-6, and IL-4 were measured by RT-PCR. (C) The levels of each mRNA were normalized to the levels of GAPDH. Densitometric analysis was performed by using Image J software. (D) The protein expressions of caspase-1 in the skin lesion homogenate were analyzed using western blotting. (E) The relative intensities were quantified based on the intensities of GAPGH bands. (F) Caspase-1 activity in the skin lesions homogenate was assessed by means of a caspase-1 assay kit. $^{\#}p < 0.05$ compared with vehicle group. $^{*}p < 0.05$ compared with DNFB control group.

Table 2
Effects of *p*CA on levels of histamine, IgE, and inflammatory cytokines in serum of DNFB-induced AD-like mouse model

	B	-	*p*CA (50 mg/kg)	*p*CA (50 mg/kg)	DEX (10nM)
	-	DNFB	-	DNFB	DNFB
Histamine (ng/ml)	8.97 ± 0.25	15.87 ± 2.25#	8.85 ± 0.29	10.33 ± 0.09*	9.88 ± 0.26*
IgE (ng/ml)	2.58 ± 0.08	35.98 ± 1.67#	2.45 ± 0.04	28.76 ± 0.77*	30.54 ± 1.87*
TSLP (ng/ml)	0.03 ± 0.00	0.60 ± 0.06#	0.03 ± 0.00	0.38 ± 0.05*	0.49 ± 0.01*
TNF-α (ng/ml)	0.91 ± 0.02	1.42 ± 0.08#	0.92 ± 0.01	1.01 ± 0.07*	1.01 ± 0.04*
IL-6 (ng/ml)	6.20 ± 0.13	10.49 ± 0.74#	6.50 ± 0.12	7.98 ± 0.31*	7.90 ± 0.42*
IL-4 (ng/ml)	0.50 ± 0.01	0.82 ± 0.06#	0.49 ± 0.02	0.67 ± 0.00*	0.59 ± 0.03*

The levels of IgE, TSLP, TNF-α, IL-6, and IL-4 in serum were measured by using ELISA. The levels of histamine in serum were determined by *o*-phthaldialdehyde spectrofluorometric procedure. #$p < 0.05$ compared with vehicle group. *$p < 0.05$ compared with DNFB control group.

elevation in HMC-1 cells. Our preliminary experiment showed that exposure to PMACI increased the mRNA expression and production of TSLP in HMC-1 cells [12]. Deficiency of TSLP ameliorated skin inflammation in a murine AD model [34]. Intradermal injection of recombinant TSLP increased scratching behavior in a murine AD model [35]. TSLP expression was elevated in lesional skin from AD patients [36]. Our results presented that the protein and mRNA expression levels of TSLP in mast cells, splenocytes, and serum were downregulated by *p*CA treatment (Figs. 1C and D, 4A-4C; Tables 1 and 2). Hence, we can assume that *p*CA might be beneficial to treat atopic and inflammatory disorders. Also, cytokine TNF-α, IL-1β, and IL-4 increased in AD patients [37]. IL-6 levels increased in skin lesions of AD patients [38]. Higher levels of IFN-γ showed in AD patients in comparison with control subjects [39]. In the present study, *p*CA improved the levels of TNF-α, IL-1β, IL-6, IL-4, and IFN-γ, validating a potential of *p*CA in AD.

Calcium chelator BAPTA-AM reduces RIP2 expression in HMC-1 cells, suggesting that calcium is an upstream regulator of RIP2 [40]. RIP2 induces caspase-1 activation by promoting its oligomerization [41]. Treatment with pCA prevented the increase of intracellular calcium in HMC-1 cells (Fig. 1B). Thus, the results of the present study suggest that *p*CA may downregulate TSLP levels through blocking of calcium/RIP2/caspase-1 signaling in HMC-1 cells.

In general, activation of caspase-1 resulted from pro-inflammatory stimuli [41]. Many studies suggest that activation of caspase-1 resulted from pro-inflammatory stimuli such as PMACI [42–44]. Kakeda et al [45] reported that AD-like skin lesions were spontaneously induced in caspase-1 overexpressing mice. *p*CA down-regulated the activation of caspase-1 in HMC-1 cells and DNFB-induced skin lesions (Figs. 2 and 4), suggesting that *p*CA may reduce TSLP by blockade of caspase-1 activation in AD.

Deficiency of RIP2 produces a decrease in phosphorylation of p38, JNK, and ERK [46]. Caspase-1 increases p38 phosphorylation independent on NF-κB activation [47]. We showed that *p*CA reduces phosphorylation of p38, JNK, and ERK in HMC-1 cells (Fig. 2D and E). Thus, we presuppose that downregulation of TSLP levels by *p*CA, at least in part, might be regulated by RIP2/caspase-1/MAPK pathways.

It has been reported that TSLP is controlled by NF-κB [48]. Previous study demonstrated that mRNA expression and production of TSLP are regulated by NF-κB in HMC-1 cells [12]. This study presented that phosphorylation of IKKβ and IκBα as well as activation of NF-κB are reduced *p*CA treatment (Fig. 2F and G). In addition, a critical transcription factor for TSLP expression is NF-κB [49]. Hence, we suggest that *p*CA would downregulate TSLP levels *via* blocking of NF-κB.

Finally, epidermal thickening and inflammatory cells infiltration are histological features of AD in human [50]. AD-like skin lesions, epidermal thickening, and inflammatory cells infiltration were reduced by *p*CA administration (Fig. 3). Choi and colleagues reported that an infiltration of mast cell into skin lesions is elevated in AD, presenting a role of mast cells in AD [51]. Oral administration of *p*CA decreased an infiltration of mast cell into skin lesions (Fig. 3B and C). Serum histamine levels in AD patients were elevated compared with healthy controls [52]. Anti-histamine therapy results in an improvement of AD symptoms, with a remarkable amelioration in pruritus [52]. Administration of *p*CA produced a decrease in serum histamine levels and scratching behaviors (Table 2; Fig. 3D). Therefore, we presuppose that *p*CA may be useful to reduce histamine in AD.

Conclusionally, our study showed that treatment with *p*CA attenuated production and mRNA expression of TSLP, IL-6, IL-1β, and TNF-α, in HMC-1 cells. *p*CA downregulated intracellular calcium levels, activation of RIP2 and caspase-1, phosphorylation of p38, JNK, and ERK, activation of NF-κB, as well as phosphorylation of IKKβ and IκBα in HMC-1 cells (supplementary fig. 2). Additionally, *p*CA decreased production of TSLP, TNF-α, IL-6, IL-4, and IFN-γ in the supernatant of stimulated splenic cells. Treatment with *p*CA alleviated AD-like skin lesions and decreased the protein and mRNA levels of TSLP, IL-6, and IL-4, as well as caspase-1 activation in the skin lesions. Histamine, IgE, TSLP, TNF-α, IL-6, and IL-4 levels were suppressed by *p*CA in the serum. Our results suggest that *p*CA can serve as a potent therapeutic agent for atopic and inflammatory disorders via downregulation of TSLP.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2020.06.004.

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