

Original article

Dehydrocostus lactone, a sesquiterpene from *Saussurea lappa* Clarke, suppresses allergic airway inflammation by binding to dimerized translationally controlled tumor protein

Haejun Pyun, Unwoo Kang, Eun Kyoung Seo, Kyunglim Lee*

Graduate School of Pharmaceutical Sciences, College of Pharmacy, Ewha Womans University, Seoul 03760, Republic of Korea

ARTICLE INFO

Keywords:

Allergic diseases
Dehydrocostus lactone
Dimerized TCTP
Histamine releasing factor
Saussurea lappa Clarke

ABSTRACT

Background: We previously reported that the biologically active form of histamine releasing factor (HRF) is dimerized translationally controlled tumor protein (dTCTP) which is involved in a number of allergic diseases. **Hypothesis/purpose:** Hoping that agents that modulate dTCTP may provide new therapeutic targets to allergic inflammatory diseases, we screened a library of natural products for substances that inhibit dTCTP. One such inhibitor we found was dehydrocostus lactone (DCL), a natural sesquiterpene present in rhizome of *Saussurea lappa* Clarke, the subject of this study.

Methods: We evaluated the therapeutic efficacy of DCL in a mouse model of ovalbumin (OVA)-induced allergic airway inflammation, employing the ELISA system using BEAS-2B cells and splenocytes, and confirmed that DCL interacts with dTCTP using SPR assay.

Results: DCL inhibited dTCTP-induced secretion of IL-8 in BEAS-2B cells. From kinetic analysis of dTCTP and DCL, we found that K_D value was $5.33 \pm 0.03 \mu\text{M}$ between dTCTP and DCL. DCL also significantly reduced inflammatory lung eosinophilia, type 2 cytokines in BALF, as well as OVA specific IgE and mucus production in a mouse model of ovalbumin induced allergy. Moreover, DCL suppressed NF- κ B activation.

Conclusion: DCL's therapeutic potential in allergic airway inflammation is based on its anti-inflammatory activity of suppressing the function of dTCTP.

Introduction

Translationally controlled tumor protein (TCTP), also known as histamine releasing factor (HRF), p21, p23, and fortilin, is ubiquitously expressed in eukaryotic groups, and is highly conserved in species ranging from humans to alfalfa plants (Bommer and Thiele, 2004). Intracellular TCTP has been variously reported to act as a microtubule-stabilizing protein (Yarm, 2002), an anti-apoptotic protein (Li et al., 2001), and a suppressor of Na, K-ATPase (Jung et al., 2004). Extracellular TCTP has also been reported to act as a histamine-releasing factor hence its name as HRF (MacDonald et al., 1995). The potential clinical roles of HRF in allergic inflammatory responses have been reported in many studies. Increased HRF was observed in skin blister fluids of patients with late phase allergic inflammation (Warner et al., 1986) and in bronchoalveolar lavage fluids of patients with idiopathic eosinophilic pneumonia and asthma (Kazuo et al., 2003). TCTP was also found responsible for spontaneous release of histamine in patients

with atopic dermatitis and food hypersensitivity (Sampson et al., 1989). Our group has shown that active form of HRF, participating in allergic inflammation, is dimerized TCTP (dTCTP) (Kim et al., 2009). Our prior studies led us to investigate whether modulation of dTCTP's role in the release of inflammatory or allergic mediators can provide an approach to the therapy of allergic inflammation diseases.

We therefore screened the natural products library for substances that inhibit dTCTP and came upon dehydrocostus lactone (DCL), a natural sesquiterpene lactone present in rhizome of *Saussurea lappa* Clarke (Robles et al., 1995). We found that many studies have already been reported that DCL exhibits a variety of biological activities, including playing roles as an anti-inflammatory (Lee et al., 1999), immunomodulatory (Taniguchi et al., 1995), anti-bacterial, and pro-apoptotic agent, and also in inhibitory action of NF- κ B activation (Jin et al., 2000), Nrf2 expression (Park et al., 2014), MAPK activation (Hsu et al., 2009) and STAT3 activation (Kuo et al., 2009). We decided to investigate whether DCL modulates dTCTP's role in the release of

Abbreviations: BALF, bronchoalveolar lavage fluid; DCL, dehydrocostus lactone; dTCTP, dimerized translationally controlled tumor protein; ELISA, enzyme-linked immunosorbent assay; HRF, histamine releasing factor; NF- κ B, nuclear factor kappa B; OVA, ovalbumin; SPR, surface plasmon resonance

* Corresponding author.

E-mail address: klyoon@ewha.ac.kr (K. Lee).

<https://doi.org/10.1016/j.phymed.2018.03.045>

Received 1 October 2017; Received in revised form 16 January 2018; Accepted 18 March 2018
0944-7113/ © 2018 Elsevier GmbH. All rights reserved.

inflammatory or allergic mediators, employing a mouse model of ovalbumin (OVA)-induced allergic airway inflammation.

Methods

Preparation of recombinant dTCTP

We prepared this recombinant protein as previously described (Kim et al., 2009). *Escherichia coli* BL21 (DE3) pLysS cells were transformed with PRSET A/mutated TCTPs (dTCTP), and the overexpressed dTCTP was purified using a Ni²⁺-charged His-Bind column according to manufacturer's protocol (ELPIS, Daejeon, Korea). The NH₂-terminal fusion proteins of TCTP (dTCTP) were separated by fast protein liquid chromatography on a High trap Q column (GE healthcare, Piscataway, NJ, USA) using a NaCl gradient.

Cell viability studies

Cell viability was measured using a Cell Counting Kit-8 (CCK) assay (Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. BEAS-2B cells (1×10^4 cells/well) were incubated in 96 well plates with various concentrations of acetone extracts of *Saussurea lappa* as well as costunolide and DCL for 24 h. CCK-8 reagent was added to each well, followed by incubation for additional 2 h, and the absorbance was measured at 450 nm using a microplate reader (MOLECULAR DEVICES, Sunnyvale, CA, USA). The percentage of viable cells was calculated using the equation: cell viability (%) = (mean absorbance in test wells/mean absorbance in control wells) $\times$ 100.

Measurement of IL-8

Cell culture for measuring IL-8 was carried out in the same manner as in the previous experiment (Kim et al., 2009). The indicated amounts (0, 1, 2, 4 μ g/ml) of DCL and 8 μ g/ml of dTCTP were pre-incubated for 15 min, added to cells in 1% penicillin-streptomycin/BEBM, and incubated for 20 h. IL-8 secreted into medium was measured by ELISA using a commercial kit (Biolegend, San Diego, CA, USA). The same method was applied to the acetone extract of *Saussurea lappa* (0, 0.25, 0.5, 1, 2 μ g/ml) and the costunolide (0, 0.125, 0.25, 0.5, 1 μ g/ml).

Surface plasmon resonance (SPR)

Binding of DCL to dTCTP was measured using a Reichert SR7500DC instrument (Reichert Technologies, Depew, NY, USA). dTCTP in 10 mM sodium acetate buffer pH 5.0 was immobilized using the standard amino coupling at 15 μ l/min on a 500,000 Da carboxymethyl dextran hydrogel surface sensor chip (Reichert Technologies, Depew, NY, USA). The running buffer used in all experiments was PBS pH 7.4 (2% DMSO). All SPR experiments were performed at 25 °C. DCL (6.25, 12.5, 25, 50, 100, 200 μ M) was injected over the dTCTP chip at 30 μ l/min for 5 min. Complete dissociation of DCL and dTCTP was achieved after 8 min. The binding of DCL and dTCTP was detected as a change in the refractive index at the surface of the chip as measured by response units (RU). A reference flow cell was used to record the background response, and background was subtracted from each of the measured RU values. K_d values were calculated as ratios of K_a/K_d determined from kinetic experiments. Data model were fits using SCRUBBER-2. This experiment was carried out by WOOJUNG BSC (Suwon, Korea).

Animals

Female BALB/c mice (5–6 weeks and weighing 18–20 g) were purchased from the Central Lab. Animal Inc (Seoul, Korea). Before the experiments, the mice were allowed to become accustomed to their new environments for 1 week and were supplied with standard rodent feed and tap water. The animal room was maintained at 60–80% relative

humidity at room temperature under a 12/12 h light/dark cycle. All animal studies were approved by Ewha Womans University's Institutional Animal Care and Use Committee (Approval ID: 16-023).

Measurement Th2 cytokines in murine splenocytes

To obtain splenocytes, the spleen was removed from the mouse (BALB/c, 6 weeks old) aseptically. Homogenized purified splenocytes were collected and treated with red blood cell lysing buffer (Sigma, St. Louis, MO, USA). The splenocytes were diluted to 7×10^6 cells/ml in RPMI 1640 media containing 10% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin. The splenocytes were cultured with DCL (0, 1, 2, 4 μ g/ml) after treatment with OVA (100 μ g/ml) or cultured without OVA and DCL treatment. The plates were incubated at 37 °C for 72 h in a humidified incubator with 5% (v/v) CO₂ and 95% (v/v) air. IL-4 and IL-5 in the supernatants of splenocytes were measured using mouse IL-4 (Biolegend, San Diego, CA, USA), IL-5 (Thermo, Rockford, IL, USA) ELISA kits.

Mouse model of OVA-induced allergic airway inflammation

Female BALB/c mice, 6–7 weeks of age, were injected intraperitoneally, with 100 μ l (0.5 mg/ml) of OVA (Thermo, Rockford, IL, USA) conjugated with same amount of alum (Thermo, Rockford, IL, USA), on days 1, and 14. Two weeks after the second injection, the animals were challenged with 20 μ l of saline (sham) or 100 μ g of OVA injected into the airway via their nasal cavity on days 28, 30, 32, 34 days. Saline (sham), saline containing 2% DMSO (vehicle), Dexamethasone (1 mg/kg) or DCL (2.5 mg/kg, 5 mg/kg, 10 mg/kg) was injected intraperitoneally into each of 3–6 mice from day 28 to day 35. Dexamethasone and DCL were purchased from Sigma (St. Louis, MO, USA).

Bronchoalveolar lavage fluid (BALF) collection and eosinophil count

Mice were anaesthetized and the trachea was cannulated while the thorax was gently massaged. Lungs were lavaged three times with 0.6 ml of phosphate buffer. The collected lavage fluid was cooled on ice and was centrifuged at 1000g for 5 min at 4 °C. The supernatant was stored at –80 °C for ELISA assays. The pellets were resuspended in 0.1 ml in phosphate buffer, and the total inflammatory cell numbers and differential counts were assessed by hemavet (Drew Scientific Inc., Oxford, CT, USA).

Enzyme-linked immunosorbent assay (ELISA)

IL-4, IL-5, IL-13, IFN- γ in BALF were measured using specific mouse IL-4 (Biolegend, San Diego, CA, USA), IL-5 (Thermo, Rockford, IL, USA), IL-13 (Biolegend, San Diego, CA, USA), IFN- γ (Biolegend, San Diego, CA, USA) ELISA kits. Serum OVA specific IgE also was measured using an ELISA kit (Biolegend, San Diego, CA, USA). All ELISAs were performed according to the manufacturer's directions.

Histological analysis

Standard procedures (Lin et al., 2014) were employed for fixation, preparation of tissue sections, deparaffination, HE and PAS stains. The densities of total inflammatory cells in the peribronchial areas of mice from different groups, and mucus occlusion of airway diameter were assessed as described previously (Henderson et al., 2005).

Lung protein extraction and western blot analysis

Lung tissue was homogenized with 500 μ l lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 0.25% deoxycholate, 1% triton X-100, phosphatase inhibitor 2, 3, protease inhibitor), incubated for

30 min, and then vortexed with tissue lyser II (QIAGEN, Hilden, Germany) for 2 min and was centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatant was stored at –70 °C until use. Proteins (10 µg) from lung tissue were suspended in sample buffers (1 M Tris–HCl, 10% SDS, 100% glycerol, 1% BPB, β-mercaptoethanol) boiled for 10 min, and separated by 10% SDS PAGE electrophoresis. The protein was then transferred electrophoretically to NC membranes (GE healthcare, Piscataway, NJ, USA). The blotted membranes were blocked with 5% skim milk in TBS buffer (10 mM Tris–HCl, 150 mM NaCl) at a room temperature for 1 h and then probed with antibodies to anti-IkBα, anti-phospho-IkBα, anti-GAPDH at 4 °C overnight. Antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). The blots were then washed 3 times with TTBS buffer (10 mM Tris–HCl, 150 mM NaCl, 0.05% tween 20) and incubated with appropriate horseradish peroxidase conjugated secondary antibodies at room temperature for 1 h. The membranes were finally washed 3 times, and signals were developed using enhanced chemiluminescent (ECL) reagent (Amersham Bioscience, Freiburg, Germany) and UV Products Imaging system, LAS 3000 (Fuji, Japan) according to manufacturer's instructions.

Statistical analysis

All results are expressed as mean ± standard error of mean (S.E.M.). Statistical analysis was performed using the prism statistical analysis program (GraphPad 5.01). Dunnett's test was used for statistical comparisons of multiple group ($n > 3$). $p < 0.05$ was considered significant for all test. Statistical significance was presented as * $p < 0.05$; ** $p < 0.01$.

Results and discussion

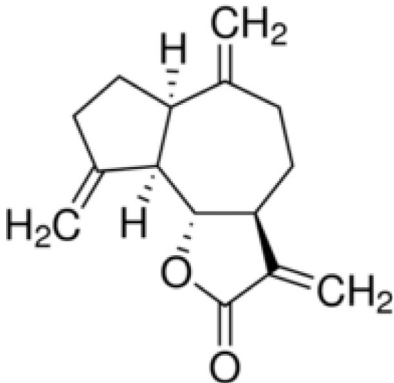
Search for substances that modulate the biological functions of dTCTP

We generated and screened a natural products library that for substances that inhibit or modify the functions of dTCTP. We found that DCL, a natural sesquiterpene lactone found in the acetone extracts of rhizome of *Saussurea lappa Clarke*, inhibited dTCTP among a variety of other biological activities, noted earlier. The structure and chemistry of this compound, gathered from the literature, are shown in Table 1. DCL

Table 1

Some Physico-Chemical data on dehydrocostus lactone.

(3aS,6aR,9aR,9bS)-Decahydro-3,6,9-tris(methylene)-azuleno [4,5-*b*] furan-2(3H)-one.

Molecular formula & characteristics	Structural formula
Empirical formula: C ₁₅ H ₁₈ O ₂	
Molecular weight: 230.30 CAS number: 477-43-0 MDL number: MFCD00210277 PubChem CID: 73174	

can be found in together with costunolide (Butturini et al., 2011; Ueda et al., 2013)

DCL inhibits IL-8 release induced by dTCTP

We compared the inhibitory effects of DCL, costunolide and acetone extract of *Saussurea lappa Clarke* on dTCTP-induced secretion of IL-8 in BEAS-2B cells. First, we tested the dose dependent effects on cell viability of these three materials (Fig. 1A–C). Cell viability after DCL treatment was greater than that after treatment with the other two substances. Next, we examined dTCTP-induced secretion of IL-8 in BEAS-2B cells. Each of the three substances were used in the concentration range that cytotoxicity was not observed. All three substances decreased dTCTP-induced secretion of IL-8 in BEAS-2B cells in dose dependent manners (Fig. 1D–F). DCL appeared to have the lowest toxicity and was therefore further deemed to have the best potential to serve as a suppressor of dTCTP-induced allergic inflammation.

DCL binds to dTCTP

TCTP has been reported to bind to anti-histamine agents, such as promethazine and hydroxyzine, and show anti-cell proliferative effects (Tuynder et al., 2004). TCTP has also been reported to bind to levomepromazine (K_D : $57.2 \pm 6.49 \mu\text{M}$) and buclizine (K_D : $433 \pm 47.1 \mu\text{M}$) (Seo and Efferth, 2016). Based on these results, we hypothesized that DCL exerts its anti-allergic activity by directly interacting with dTCTP. Kinetic analysis of the interaction between dTCTP and DCL confirmed this hypothesis. The interaction between dTCTP and DCL was also measured using surface plasmon resonance spectrometry. The equilibrium dissociation constant (K_D) of DCL for dTCTP was $5.33 \pm 0.03 \mu\text{M}$ (Table 2). This confirms that the direct binding of DCL to dTCTP inhibits histamine releasing activity of dTCTP.

DCL inhibits inflammatory cytokine release in primary cultured splenocytes sensitized by OVA

Cytokines such as IL-4 and IFN-γ can be found in the supernatants of stimulated splenocytes (Xiang et al., 2015). In supernatants of splenocytes of the asthma mouse model, the IL-4 level was increased and IFN-γ level was decreased compared to the control (Xiang et al., 2015). In our study, mouse splenocytes sensitized by OVA were treated with DCL, and the changes in type 2 cytokines directly associated with allergic inflammation were examined (Wynn, 2015). We found that DCL inhibits IL-4 and IL-5 release in the supernatants of the mouse splenocytes dose-dependently (Fig. 2A and B). This suggests that DCL exerts its anti-allergic effect by inhibiting cytokines secreted by the splenocytes.

It is known that when cells such as macrophages are stimulated, they secrete intracellular TCTP, which acquires cytokine-like activity by altering its tertiary structure by dimerization (Kim et al., 2013). To examine if this is true in the case of the OVA sensitized splenocytes of our animal model, we investigated the presence of dTCTP in the supernatant of OVA sensitized splenocytes. dTCTP was found in the supernatant of OVA sensitized splenocytes, but not in the control (Fig. 2C). It can be inferred that splenocytes sensitized by OVA secrete dTCTP which in turn stimulates the splenocytes to secrete type 2 cytokines associated with allergic reactions. As shown in Fig. 2C, there were no difference in the amounts of dTCTP secreted in OVA stimulation group and the OVA with DCL stimulation group, but cytokine release was significantly reduced in DCL group. Since the dTCTP level did not change, but the cytokine level decreased, we speculate that the DCL binds to dTCTP and inhibits its activity thereby inhibiting the stimulation of splenocytes.

DCL reduces inflammatory cell influx in BALF

Eosinophils are the major inflammatory cells present in the airways

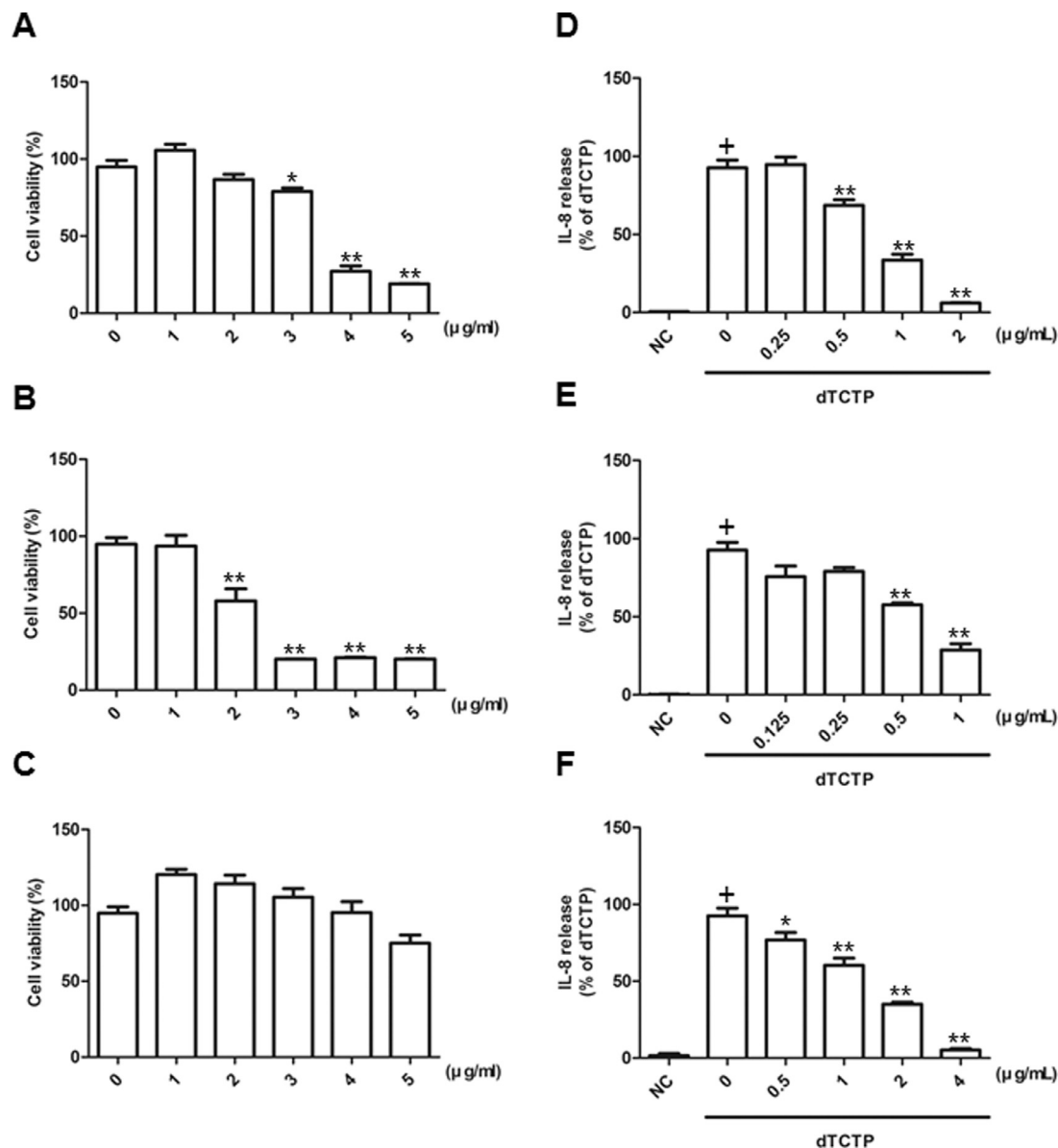


Fig. 1. Cytotoxicity of *Saussurea lappa Clarke* acetone extracts (A), costunolide (B) and DCL (C) in BEAS-2B cells. Cells were seeded into 96 well plates and treated with various concentrations of each substance for 24 h. Cell viability was assessed using a CCK-8 assay. The ability of *Saussurea lappa Clarke* acetone extract (D), costunolide (E) and DCL (F) to inhibit IL-8 secreted by dTCTP in BEAS-2B cells. The indicated microgram of each substance was premixed with 8 μg/ml TCTP for 15 min. After 20 h, the supernatants were harvested and their IL-8 levels were measured. The standard range of IL-8 ELISA kit was 15.6–1000 pg/ml. These values are expressed as the mean $\pm$ SEM. $n = 3$, $+p < 0.05$ compared with the negative control; $*p < 0.05$, $**p < 0.01$ compared with the only dTCTP treatment group.

Table 2

Kinetic binding parameters for dehydrocostus lactone. Dehydrocostus lactone binding to dTCTP determined by SPR (Reichert SPR SR7500 instrument).

Substance	Ligand	Kinetic binding parameters		
		K_a ($M^{-1} s^{-1}$)	K_d (s^{-1})	K_D (μM)
Dehydrocostus lactone	Dimer TCTP	52.5 ± 0.3	$2.80e^{-4} \pm 0.03e^{-4}$	5.33 ± 0.03

Numbers in parentheses represent the standard error of the kinetic fit.

that contribute to the pathogenesis of asthma (Eum et al., 2003). Our data show that total number of inflammatory cells and the differential count of eosinophils in BALF samples significantly decreased in OVA sensitized mice injected with DCL in a dose dependent manner, compared to OVA sensitized mice injected with the vehicle (Fig. 3). Treatment with DCL significantly decreased eosinophil and

inflammatory cell influx, suggesting that DCL attenuated OVA induced allergic airway inflammation.

DCL regulates level of inflammatory cytokines in BALF

Activation of allergen-specific T helper 2 cells induces type 2 cytokines, which induce B cells and promote eosinophil maturation (Jarnicki, 2003). Type 2 cytokines such as IL-4, 5, and 13 play an important role in the initiation and progression of allergic disorders (Oliphant et al., 2011). IL-4 promotes initial differentiation of Th2 cells, B cell activation, IgE synthesis and eosinophil recruitment (Brusselle et al., 1994). IL-5 is responsible for promoting proliferation and influx of eosinophils while IL-4 and IL-13 play a role in converting B cells to produce IgE (Barnes, 2008). IL-13 is involved in allergic airway hyper responsiveness (AHR), B cell activation and airway remodeling including goblet cell hyperplasia and infiltration of inflammatory cells such as eosinophils (Chung and Barnes, 1999). In the

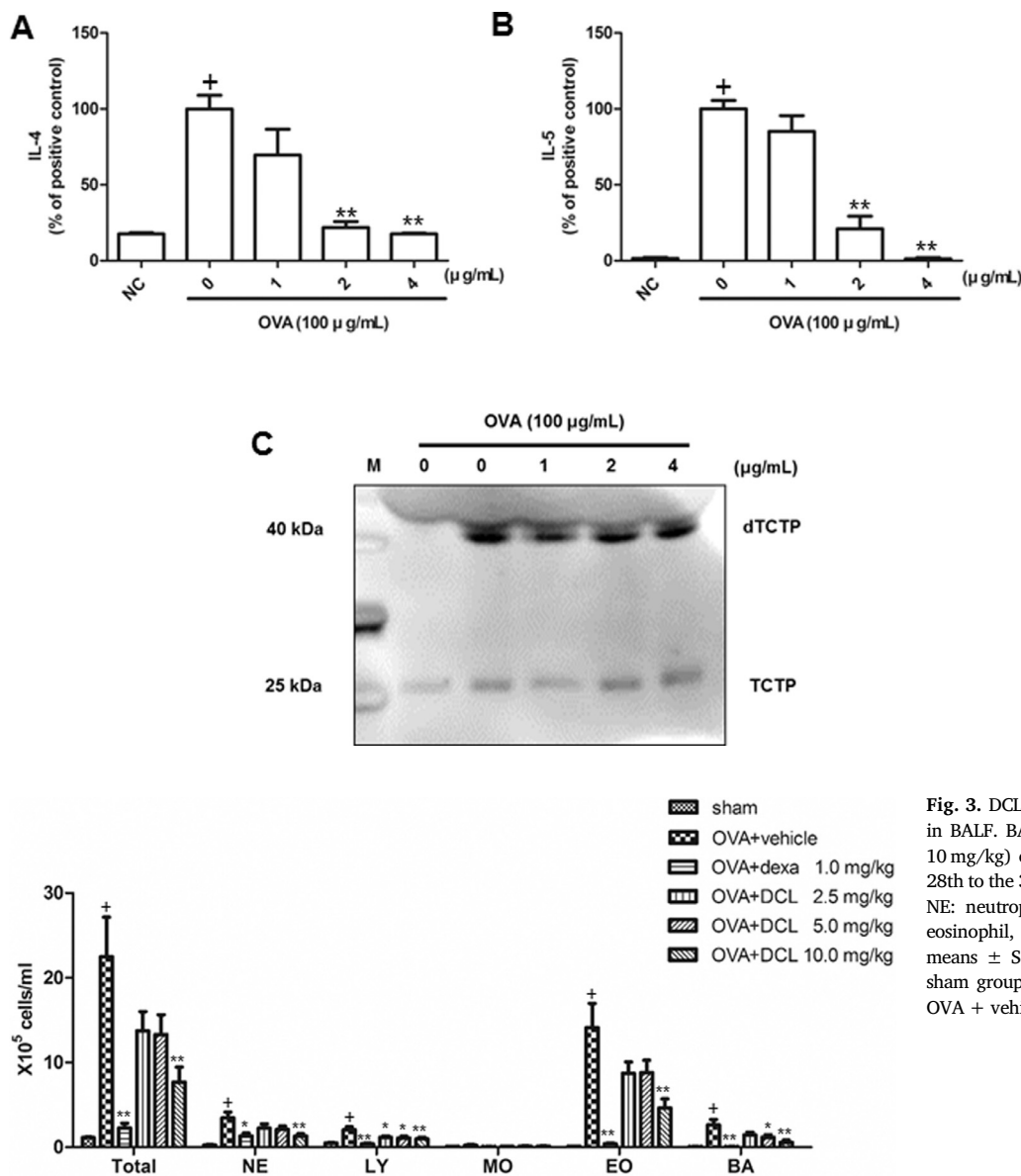


Fig. 2. DCL suppresses OVA-induced IL-4, IL-5 production. After sensitization of mice with OVA, homogenized spleen cells were incubated for 48 h with DCL in the presence of OVA, and culture media was analyzed by ELISA to detect levels of IL-4 (The Standard range was 2.0–125 pg/ml)(A), IL-5 cytokines (The standard range was 7.8–320 pg/ml)(B). To confirm the changes of d TCTP in the culture media of splenocytes, dTCTP was detected in the culture medium by western blotting (C). These values are expressed as the mean $\pm$ SD. $n = 3$, $+ p < 0.05$ compared with the negative control; $*p < 0.05$, $**p < 0.01$ compared with the only OVA sensitization group.

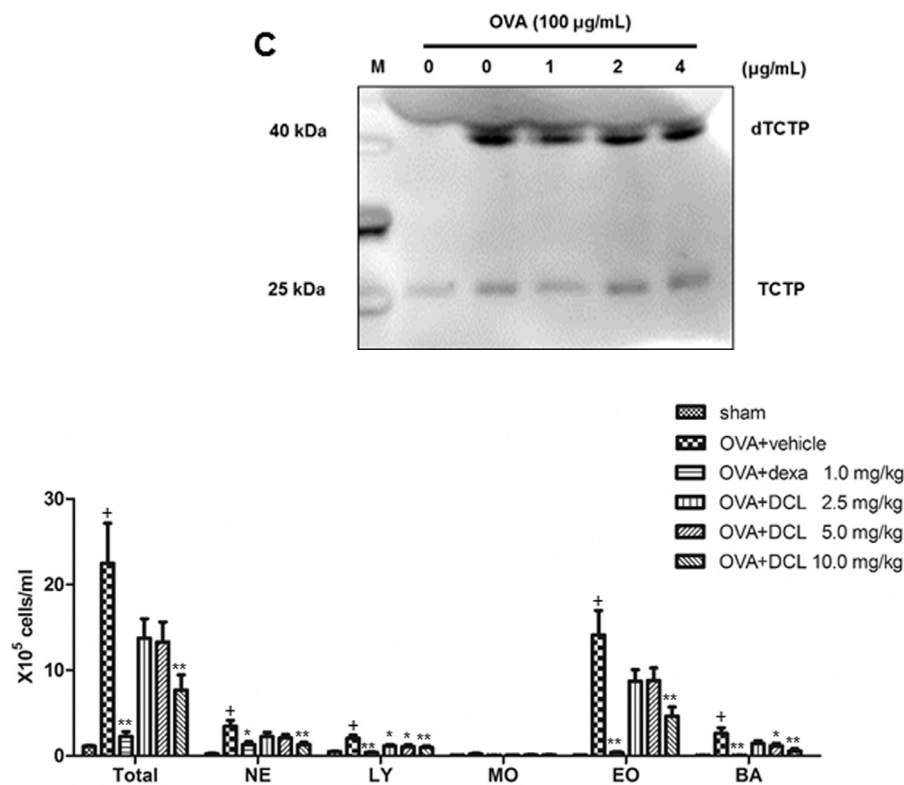


Fig. 3. DCL decreases recruitment of inflammatory cells in BALF. BALB/c mice were treated with DCL (2.5, 5, 10 mg/kg) or vehicle (1% DMSO in saline) daily from 28th to the 35th day after the first immunization of OVA. NE: neutrophil, LY: lymphocyte, MO: monocyte, EO: eosinophil, BA: basophil. The values presented are means $\pm$ SEM. $n = 3-6$, $+ p < 0.05$ compared with the sham group; $*p < 0.05$, $**p < 0.01$ compared with the OVA + vehicle group.

mouse model of allergic airway inflammation, type 2 cytokines including IL-4, IL-5, IL-13 are specifically increased by OVA sensitization and challenge (Holgate, 2008). In our study, we discovered that DCL down-regulated OVA induced type 2 cytokines in a dose dependent manner (Fig. 4A–C).

IFN- γ , a representative Type 1 cytokines, has been reported to attenuate allergy-induced airway inflammation and AHR in mice (Yao et al., 2016). The imbalance between type 1 and type 2 cytokines causes asthma associated with upregulated Th2 cytokine production, decreased Th1 cytokine production, increased IgE and airway eosinophilic inflammation (Busse and Lemanske, 2001). To determine whether DCL affects the balance of type 1 and type 2 cytokines, IFN- γ was measured in BALF by ELISA. IFN- γ level of the BALF was slightly up-regulated in OVA stimulated mice with treated with DCL (Fig. 4D). These results suggest that DCL may help to restore type 1 and type 2 cytokine balance, in a pathway that relieves allergic airway inflammation. This suggestion needs further study.

DCL reduces OVA specific IgE levels in serum

IgE is considered to be critical to the allergic response of allergic

diseases (Gould and Sutton, 2008). In the case of atopic dermatitis patients, various reactions are caused by IgE dependent mechanisms after exposure to allergen (Choi and Yan, 2009). Also, higher production of IgE favors the induction of degranulation of mast cells and airway constriction (Choi and Yan, 2009). Also it has been reported that allergic inflammation is reduced by the suppression of antigen specific IgE antibodies (Zhou et al., 2014).

We therefore examined whether DCL can regulate IgE, which plays a central role in allergic reactions. We measured OVA specific IgE level in serum of mice receiving OVA sensitization and challenge. We found that DCL reduced the IgE mediated allergic response (Fig. 5). In OVA-sensitized and challenged animals, serum OVA specific IgE was significantly reduced in mice treated with 5 or 10 mg/kg DCL but not with 2.5 mg/kg DCL, compared to mice treated with vehicles (Fig. 5). Importantly, we observed a decrease of OVA specific IgE in a dose dependent manner.

DCL ameliorates histopathological changes in lung tissue

To characterize the effects of DCL on lung tissue in the OVA induced mouse allergic airway inflammation model, lung sections were stained

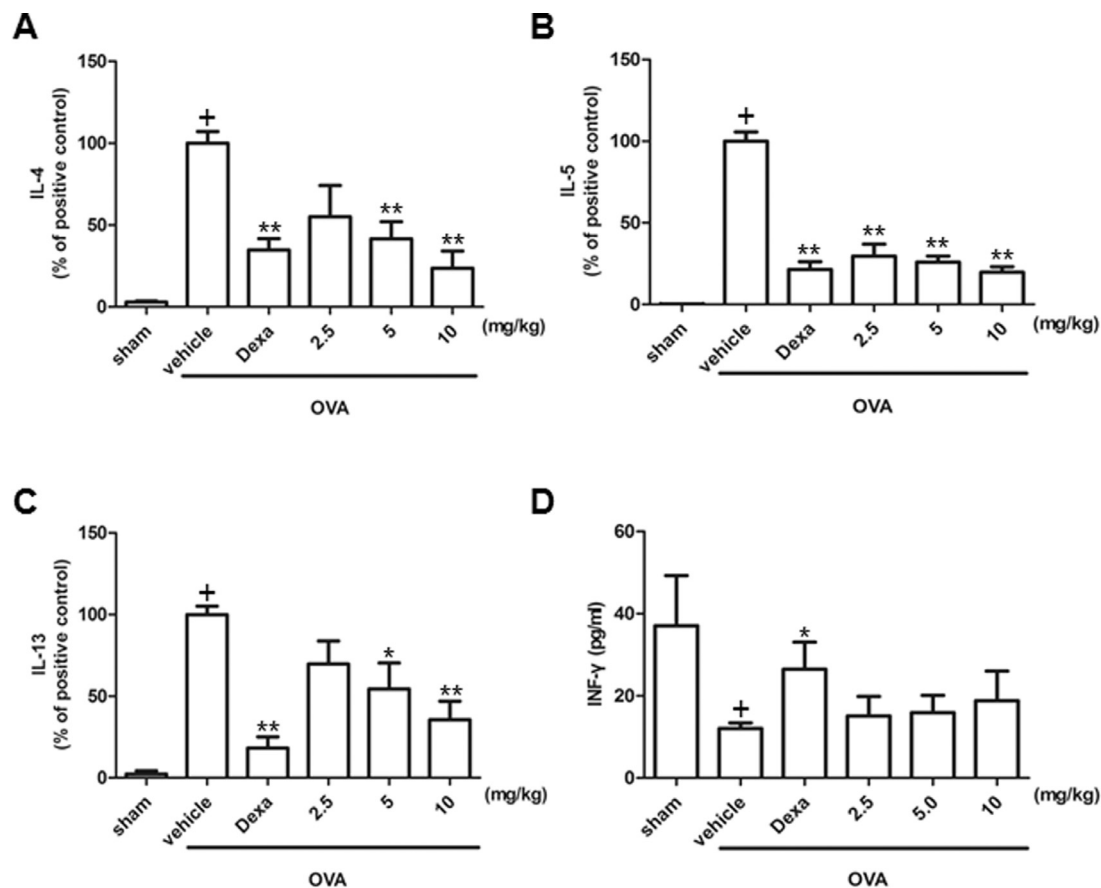


Fig. 4. DCL regulates IL-4 (A), IL-5 (B), IL-13 (C) and IFN-γ (D) in BALF. The levels of IL-4, IL-5, IL-13 and IFN-γ were detected 24 h after the last challenge. The levels of these cytokines were quantified by ELISA. The levels of IL-4, IL-5 and IL-13 increased significantly in OVA treated mice; however, the increased cytokines were inhibited by DCL. The level of IFN-γ was decreased by OVA and decrease was slightly up-regulated by the treatment with DCL. Each standard range of ELISA kits was as follows. IL-4: 2.0–125 pg/ml, IL-5: 7.8–320 pg/ml, IL-13: 15.6–1000 pg/ml, IFN-γ: 15.6–1000 pg/ml. The values presented are means $\pm$ SEM. $n = 3-6$, + $p < 0.05$ compared with the sham group; * $p < 0.05$, ** $p < 0.01$ compared with the OVA + vehicle group.

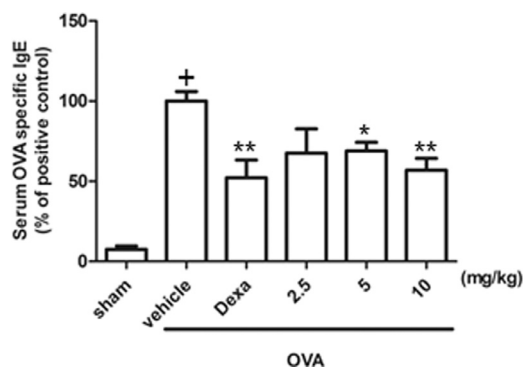


Fig. 5. DCL inhibits serum level of OVA specific IgE. The levels of OVA specific IgE was detected 24 h after the last challenge. The levels of these cytokines were quantified by ELISA. The standard range of this kit was 0.156–10 ng/ml. The Serum level of OVA specific IgE increased in mice receiving OVA sensitization and challenge. In OVA sensitized and challenged animals, OVA specific IgE was reduced in mice treated with DCL. The values presented are means $\pm$ SEM. $n = 3-6$, + $p < 0.05$ compared with the sham group; * $p < 0.05$, ** $p < 0.01$ compared with the OVA + vehicle group.

with hematoxylin and eosin and examined. As mentioned earlier, eosinophils are dominant inflammatory cells present in the airway and this is a contributing factor to the pathophysiological changes of asthma (Fulkerson and Rothenberg, 2013). The level of cell infiltration in the lungs as detected by H&E staining also decreased in allergic mice injected with DCL (Fig. 6).

Hyperplastic goblet cells stimulate the secretion of mucus into the airway lumen and promote airway remodeling (Thai et al., 2008). The effects of DCL on OVA induced airway goblet cell hyperplasia and mucus occlusion were evaluated in the lung sections after periodic acid-schiff staining. This histological study showed that DCL suppressed airway goblet cell hyperplasia, hypersecretion of mucus, and bronchiole occlusion, in a dose dependent manner (Fig. 6), all of which are characteristics of pathological changes in asthma (Holgate, 2008).

DCL inhibits the activation of the NF-κB signaling pathway

Our research group were the first to report that dTCTP activates the NF-κB pathway (Lee and Lee, 2018). NF-κB plays essential role in regulating immune responses, including the expression of genes that regulate many inflammatory cytokines, such as IL-4, IL-5 and IL-13 (Das et al., 2001). Inhibition of NF-κB is also one of the major targets of corticosteroids, which constitute the most effective asthma treatment currently available (Lin et al., 2014).

We hypothesized that DCL would inhibit the activation of NF-κB, since the binding of dTCTP and DCL was shown to inhibit the function of dTCTP. We performed western blotting to measure NF-κB activity and found that DCL inhibited the activation of NF-κB through suppressing the phosphorylation IκBα in lung tissues in our model (Fig. 7). Phosphorylation and degradation of IκB-α were effectively prevented in mice treated with DCL.

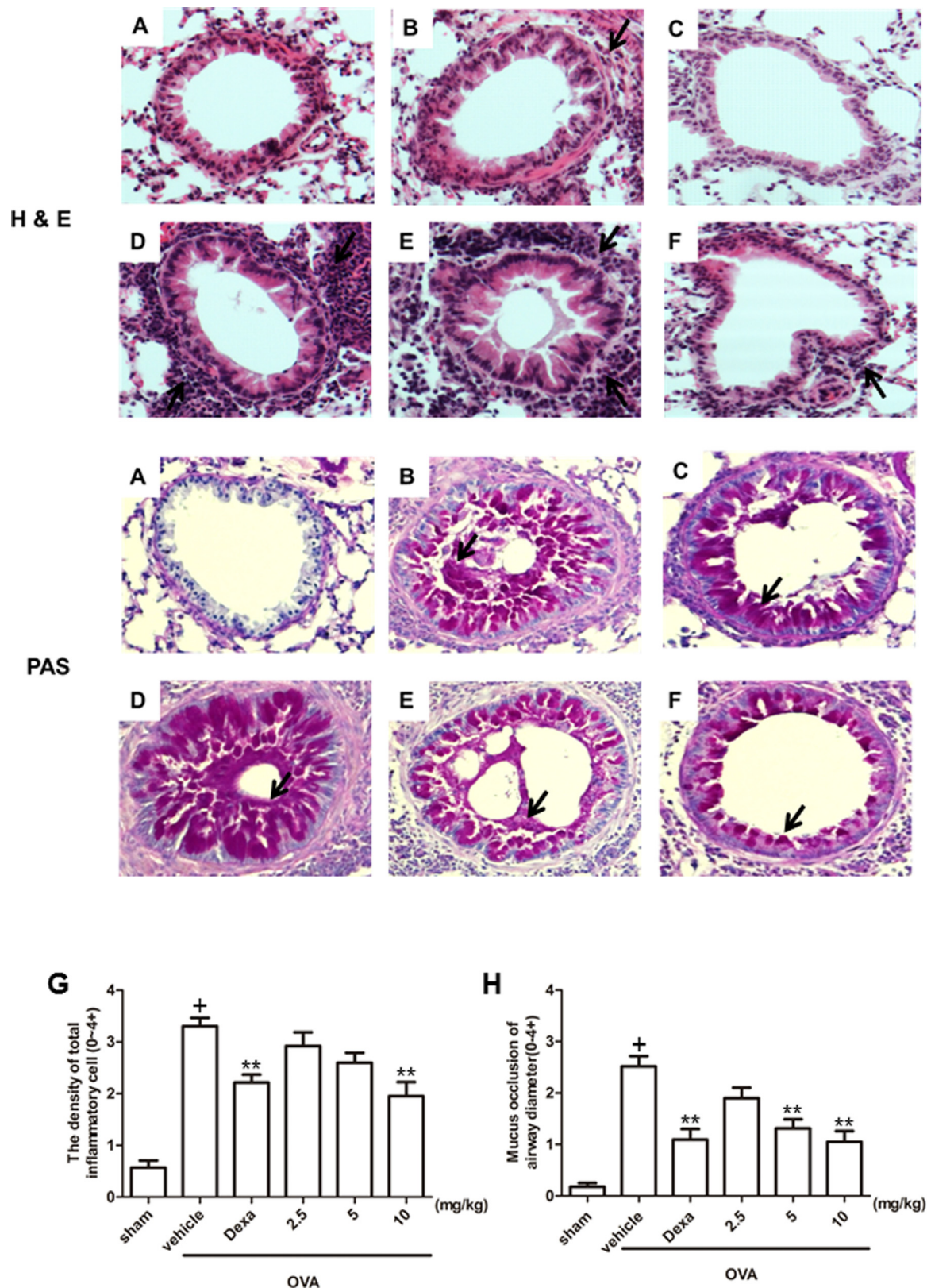


Fig. 6. The effect of DCL on histopathological changes in the lung tissues of OVA-challenged allergic airway inflammation in BALB/c mice. Lung tissues were collected 24 h after the final challenge. Inflammation was evaluated in the lung tissues by analyzing inflammatory cell infiltration (H&E) and goblet cell hyperplasia (PAS) staining. (A) Representative lung tissue sections of sham group, (B) OVA sensitized and challenged mice treated with vehicle, or (C) dexamethasone (1 mg/kg) (D–F) DCL (2.5, 5, 10 mg/kg). (G) The number of peribronchial inflammatory cell was upregulated in mice receiving OVA sensitization and challenge compare with mice receiving sham treatment. (H) Mucus occlusion was significantly attenuated in OVA sensitized and challenged mice with DCL. H&E and PAS stained sections are magnified $\times 200$. The values presented are means $\pm$ SEM. $n = 3–6$, + $p < 0.05$ compared with the sham group; * $p < 0.05$, ** $p < 0.01$ compared with the OVA + vehicle group.

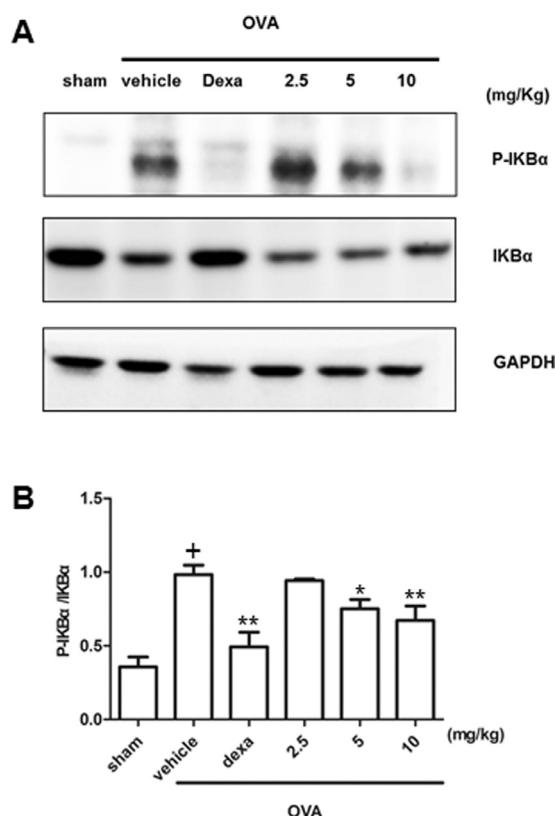


Fig. 7. The effect of DCL on NF-κB activation in OVA induced allergic airway inflammation in BALB/c mice. Lung tissue was prepared 24 h after the last OVA challenge. Western blotting detection of phosphorylated and total IκBα in lung tissue from mice of sham group, OVA sensitized and challenged mice treated with vehicle, dexamethasone (1 mg/kg) or DCL (2.5, 5 or 10 mg/kg) (A). The quantitative results were shown in (B). The values presented are means ± SEM. $n = 3-6$; + $p < 0.05$ compared with the sham group; * $p < 0.05$, ** $p < 0.01$ compared with the OVA + vehicle group.

Conclusion

DCL isolated from *Saussurea lappa* Clarke inhibited NF-κB activity by binding to dTCTP. This inhibition by DCL, resulted in the reduction of bronchial inflammation, and decreases in of the levels of type 2 cytokine, and IgE and of mucus secretion. These results suggest that DCL can be a fundamental anti-allergic agent that can alleviate allergy.

Conflicts of interest

There are no investigator conflicts of interest to declare on the part of any of the investigators.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2017R1A2B2004023) and by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (HI17C0631).

References

Barnes, P.J., 2008. Immunology of asthma and chronic obstructive pulmonary disease. *Nat. Rev. Immunol.* 8, 183–192.
 Bommer, U.-A., Thiele, B.-J., 2004. The translationally controlled tumour protein (TCTP). *Int. J. Biochem. Cell Biol.* 36, 379–385.

Brusselle, G., Kips, J., Tavernier, J., van der Heyden, J., Cuvelier, C., Pauwels, R., Bluethmann, H., 1994. Attenuation of allergic airway inflammation in IL-4 deficient mice. *Clin. Exp. Allergy* 24, 73–80.
 Busse, W.W., Lemanske, R.F., 2001. Asthma. *N. Engl. J. Med.* 344, 350–362.
 Butturini, E., Cavalieri, E., de Prati, A., Darra, E., Rigo, A., Shoji, K., Murayama, N., Yamazaki, H., Watanabe, Y., Suzuki, H., Mariotto, S., 2011. Two naturally occurring terpenes, dehydrocostuslactone and costunolide, decrease intracellular GSH content and inhibit STAT3 activation. *PLoS One* 6, e20174.
 Choi, Y.H., Yan, G.H., 2009. Ellagic acid attenuates immunoglobulin E-mediated allergic response in mast cells. *Biol. Pharm. Bull.* 32, 1118–1121.
 Chung, K.F., Barnes, P.J., 1999. Cytokines in asthma. *Thorax* 54, 825–857.
 Das, J., Chen, C.H., Yang, L., Cohn, L., Ray, P.A.R., 2001. A critical role for NF-kappa B in GATA3 expression and TH2 differentiation in allergic airway inflammation. *Nat. Immunol.* 2, 45–50.
 Eum, S.-Y., Maghni, K., Hamid, Q., Eidelman, D.H., Campbell, H., Isogai, S., Martin, J.G., 2003. Inhibition of allergic airways inflammation and airway hyperresponsiveness in mice by dexamethasone: Role of eosinophils, IL-5, eotaxin, and IL-13. *J. Allergy Clin. Immunol.* 111, 1049–1061.
 Fulkerson, P.C., Rothenberg, M.E., 2013. Targeting eosinophils in allergy, inflammation and beyond. *Nat. Rev. Drug Discov.* 12, 117–129.
 Gould, H.J., Sutton, B.J., 2008. IgE in allergy and asthma today. *Nat. Rev. Immunol.* 8, 205–217.
 Henderson Jr., W.R., Banerjee, E.R., Chi, E.Y., 2005. Differential effects of (S)- and (R)-enantiomers of albuterol in a mouse asthma model. *J. Allergy Clin. Immunol.* 116, 332–340.
 Holgate, S.T., 2008. Pathogenesis of asthma. *Clin. Exp. Allergy* 38, 872–897.
 Hsu, Y.L., Wu, L.Y., Kuo, P.L., 2009. Dehydrocostuslactone, a medicinal plant-derived sesquiterpene lactone, induces apoptosis coupled to endoplasmic reticulum stress in liver cancer cells. *J. Pharmacol. Exp. Ther.* 329, 808–819.
 Jarnicki, A., 2003. T helper type-2 cytokine responses: potential therapeutic targets. *Curr. Opin. Pharmacol.* 3, 449–455.
 Jin, M., Lee, H., Ryu, J., Chung, K., 2000. Inhibition of LPS-induced NO production and NF-kappaB activation by a sesquiterpene from *Saussurea lappa*. *Arch. Pharm. Res.* 23, 54–58.
 Jung, J., Kim, M., Kim, M.J., Kim, J., Moon, J., Lim, J.S., Kim, M., Lee, K., 2004. Translationally controlled tumor protein interacts with the third cytoplasmic domain of Na,K-ATPase alpha subunit and inhibits the pump activity in HeLa cells. *J. Biol. Chem.* 279, 49868–49875.
 Kazuo, Y., Kazuhito, R., Yoichi, N., Hiroaki, Y., Shigetada, K.-T., Saburo, S., 2003. Stimulation of human bronchial epithelial cells by IgE-dependent.pdf. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 286, 174–181.
 Kim, M., Maeng, J., Lee, K., 2013. Dimerization of TCTP and its clinical implications for allergy. *Biochimie* 95, 659–666.
 Kim, M., Min, H.J., Won, H.Y., Park, H., Lee, J.C., Park, H.W., Chung, J., Hwang, E.S., Lee, K., 2009. Dimerization of translationally controlled tumor protein is essential for its cytokine-like activity. *PLoS One* 4, e6464.
 Kuo, P.L., Ni, W.C., Tsai, E.M., Hsu, Y.L., 2009. Dehydrocostuslactone disrupts signal transducers and activators of transcription 3 through up-regulation of suppressor of cytokine signaling in breast cancer cells. *Mol. Cancer Ther.* 8, 1328–1339.
 Lee, H., Lee, K., 2018. Dimerized Translationally controlled tumor protein increases interleukin-8 expression through MAPK and NF-κB pathways in human bronchial epithelial cell line. *Cell Biosci.* 8, 13.
 Lee, H.J., Kim, H.Y., Jang, M.K., Son, H.J., Kim, K.M., Sohn, D.H., Lee, S.H., Ryu, J.H., 1999. A sesquiterpene, dehydrocostus lactone, inhibits the expression of inducible nitric oxide synthase and TNF-alpha in LPS-activated macrophages. *Planta Med* 65, 104–108.
 Li, F., Zhang, D., Fujise, K., 2001. Characterization of fortilin, a novel antiapoptotic protein. *J. Biol. Chem.* 276, 47542–47549.
 Lin, C.-H., Shen, M.-L., Zhou, N., Lee, C.-C., Kao, S.-T., Wu, D.C., 2014. Protective effects of the polyphenol sesamin on allergen-induced Th2 responses and airway inflammation in mice. *PLoS One* 9, e96091.
 MacDonald, S., Rafnar, T., Langdon, J., Lichtenstein, L., 1995. Molecular identification of an IgE-dependent histamine-releasing factor. *Science* 269, 688–690.
 Oliphant, C.J., Barlow, J.L., McKenzie, A.N., 2011. Insights into the initiation of type 2 immune responses. *Immunology* 134, 378–385.
 Park, E.J., Park, S.W., Kim, H.J., Kwak, J.H., Lee, D.U., Chang, K.C., 2014. Dehydrocostuslactone inhibits LPS-induced inflammation by p38MAPK-dependent induction of hemeoxygenase-1 in vitro and improves survival of mice in CLP-induced sepsis in vivo. *Int. Immunopharmacol.* 22, 332–340.
 Robles, M., Aregullin, M., West, J., Rodriguez, E., 1995. Recent studies on the zoopharmacognosy pharmacology and neurotoxicology of sesquiterpene lactones. *Planta Med.* 61, 199–203.
 Sampson, H., Broadbent, K., Bernhisel-Broadbent, J., 1989. Spontaneous release of histamine from basophils and histamine-releasing factor in patients with atopic dermatitis and food hypersensitivity. *N. Engl. J. Med.* 321, 228–232.
 Seo, E.-J., Efferth, T., 2016. Interaction of antihistaminic drugs with human translationally controlled tumor protein (TCTP) as novel approach for differentiation therapy. *Oncotarget* 7, 16818–16839.
 Taniguchi, M., Kataoka, T., Suzuki, H., Uramoto, M., Ando, M., Arao, K., Magae, J., Nishimura, T., Otake, N., Nagai, K., 1995. Costunolide and dehydrocostus lactone as inhibitors of killing function of cytotoxic T lymphocytes. *Biosci. Biotechnol. Biochem.* 59, 2064–2067.
 Thai, P., Loukoianov, A., Wachi, S., Wu, R., 2008. Regulation of airway mucin gene expression. *Annu. Rev. Physiol.* 70, 405–429.
 Tuynder, M., Fiucci, G., Prieur, S., Lespagnol, A., Geant, A., Beaucourt, S., Duflaut, D., Besse, S., Susini, L., Cavarelli, J., Moras, D., Amson, R., Telerman, A., 2004.

- Translationally controlled tumor protein is a target of tumor reversion. *Proc. Natl. Acad. Sci. U.S.A.* 101, 15364–15369.
- Ueda, J., Toda, Y., Kato, K., Kuroda, Y., Arai, T., Hasegawa, T., Shigemori, H., Hasegawa, K., Kitagawa, J., Miyamoto, K., Uheda, E., 2013. Identification of dehydrocostus lactone and 4-hydroxy- β -thujone as auxin polar transport inhibitors. *Acta Physiol. Plant.* 35, 2251–2258.
- Warner, J.A., Pienkowski, M.M., Plaut, M., Norman, P.S., Lichtenstein, L.M., 1986. Identification of histamine releasing factor(s) in the late phase of cutaneous IgE-mediated reactions. *J. Immunol.* 136, 2583–2587.
- Wynn, T.A., 2015. Type 2 cytokines: mechanisms and therapeutic strategies. *Nat. Rev. Immunol.* 15, 271–282.
- Xiang, Y., Yunxiao, S., Miao, L., 2015. Effect of *Lactobacillus salivarius* on Th1/Th2 cytokines and the number of spleen CD4+ CD25+ Foxp3+ Treg in asthma Balb/c mouse. *Int. J. Clin. Exp. Pathol.* 8, 7661–7674.
- Yao, J., Jiang, M., Zhang, Y., Liu, X., Du, Q., Feng, G., 2016. Chrysin alleviates allergic inflammation and airway remodeling in a murine model of chronic asthma. *Int. Immunopharmacol.* 32, 24–31.
- Yarm, F.R., 2002. Plk Phosphorylation regulates the microtubule-stabilizing protein TCTP. *Mol. Cell. Biol.* 22, 6209–6221.
- Zhou, E., Fu, Y., Wei, Z., Yang, Z., 2014. Inhibition of allergic airway inflammation through the blockage of NF- κ B activation by ellagic acid in an ovalbumin-induced mouse asthma model. *Food Funct* 5, 2106–2112.