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Inhibitory effect of Platycodi Radix on ovalbumin-induced airway inflammation in a murine model of asthma

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ABSTRACT

Asthma is a chronic inflammatory disease of the airways characterized by an associated increase in airway responsiveness. In this study, we investigated the inhibitory effect of an aqueous extract from the root of Platycodi Radix (Changkil: CK) on airway inflammation in a murine model of asthma. Mice were sensitized and challenged by ovalbumin (OVA) inhalation to induce chronic airway inflammation and airway remodeling. CK markedly decreased the number of infiltrated inflammatory cells and the levels of Th1 and Th2 cytokines and chemokines compared with those in the OVA-induced group. In addition, CK reduced OVA-specific IgE levels in bronchoalveolar lavage (BAL) fluid. Based on lung histopathological studies, inflammatory cell infiltration and mucus hypersecretion were inhibited by CK administration compared to that in the OVA-induced group. Lung weight was reduced after CK administration. Also, increased generation of ROS in BAL fluid, as well as NF-κB nuclear translocation, by inhalation of OVA was diminished by CK. Moreover, CK reduced the OVA-induced upregulation of matrix metalloproteases activity. These findings indicate that oxidative stress may play a crucial role in the pathogenesis of bronchial asthma induced by OVA and that CK may be useful as an adjuvant therapy for the treatment of bronchial asthma.

1. Introduction

Bronchial asthma is a chronic respiratory disease of the airway (Ahui et al., 2008; Lee et al., 2008). Asthma causes different phenotypes and varies with age, gender, and ethnic group. Inhaled pollutants including allergens, viruses, bacteria, fungi, tobacco smoke and ozone enhance the risk of developing asthma. These inhaled pollutants are reported to initiate allergic symptoms (Holgate, 2008; Selgrade et al., 2008). The allergic disease is characterized by various pathological features such as cough, rhinorrhea, and breathlessness (Roh et al., 2008; Nakajima and Takatsu, 2007). Airway hyper-responsiveness (AHR) involves infiltration of leukocytes into the airway wall, which is associated with increased expression of cytokines, enzymes, and adhesion molecules within the airway (Jung et al., 2008). T-helper (Th) cells are important regulators of immune and inflammatory reactions via the release of cytokines. The Th1 cytokines include tumor necrosis factor-alpha (TNF-α) and interferon-gamma (INF-γ), and the Th2 cytokines include interleukin (IL)-4, IL-5 and IL-13 (Roh et al., 2008). The specific Th1 cytokines enhance Th1 cell-mediated allergic airway inflammation, while the specific Th2 cytokines enhance induction of allergic responses in asthma (Nakajima and Takatsu, 2007; Finkelman and Vercelli, 2007). Furthermore, elevated Th2 cytokines have been reported to induce immunoglobin E (IgE) switching in B cells, replacement of mast cells and eosinophils, and inflammation in the lung tissue (Yuk et al., 2007). One of the chemokines, monocyte chemoattractant protein-1 (MCP-1), is responsible for the recruitment of differential leukocytes into the peribronchial tissue and the pathogenesis of allergic disease (Ip et al., 2006).

Inflammation can influence airway wall remodeling by inducing changes in airway structures such as epithelium, fibroblasts, myofibroblasts, smooth muscles, and microvasculature (Miller et al., 2008). Asthma is associated with thickening of the airway wall. Based on a histopathological study, increased submucosal and adventitial deposition of matrix proteins that comprise fibronectin and collagens I, III and V, as well as an increase in smooth muscle, account for much of this airway wall thickening (Holgate, 2008).

Oxidative stress is induced by a large variety of oxygen free radicals, including reactive oxygen species (ROS). An increasing amount of clinical and experimental evidence suggests that ROS play essential roles in the pathogenesis of airway inflammation (Kwak et al., 2003; Hamelmann et al., 1997; Lee et al., 2002). Eosinophils are known as the primary effector cells in the pathogenesis of asthma through the release of specific granule proteins and ROS (Rahman et al., 1996).

The induction of chronic cytokine expression and the recruitment and activation of inflammatory cells in asthma involve increased transcription of inflammatory cytokine genes, which is regulated by transcription factors (Barnes and Adcock, 1998).

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Nuclear factor (NF)-κB is one of the transcription factors that induce a variety of genes involved in immune and inflammatory responses (Blackwell and Christman, 1997; Wulczyn et al., 1996). After an inflammatory stimulus, the phosphorylation of IκB triggers its degradation and the translocation of NF-κB to the nucleus, where it induces the expression of a broad variety of genes, including cytokines (e.g., IL-1β, IL-6, and TNF-α), enzymes (including cyclooxygenase-2 and MMPs), adhesion molecules, and acute-phase proteins (Barnes and Karin, 1997). Recent studies have suggested that NF-κB activity may be involved in the pathogenesis of asthma, given that its increased activation has been demonstrated in the lung tissue following allergen challenge (Burrows et al., 1989) and in the airway epithelial cells and macrophages of asthmatic patients (Burrows, 1995). The regulation of matrix metalloproteases (MMPs) activity by active cleavage or through binding to natural inhibitors is believed to play an active role in lung fibrosis (Parks and Shapiro, 2001). MMP-2 and -9 activities and expression have been associated with the airway remodeling found in emphysema, acute respiratory distress syndrome, pulmonary fibrosis, and asthma, consistent with the breakdown of collagen type IV in the extracellular matrix (ECM) (Cataldo et al., 2003).

Changkil (CK), which is the aqueous extract from the root of Platycodi Radix cultivated for more than 20 years, has been used as a food as well as in traditional oriental medicine to treat chronic adult diseases such as bronchitis, asthma, pulmonary tuberculosis, hyperlipidemia, and hypercholesterolemia. CK also enhances some of the functions of macrophages, such as proliferation, spreading ability, phagocytosis, cytostatic activity, and NO secretion, as well as the gene expression of IL-1β, IL-6, and TNF-α (Choi et al., 2001a,b). Recent studies show that CK and the Changkil saponins (CKS) derived from CK have anti-inflammatory activities (Wang et al., 2004; Ahn et al., 2005; Kim et al., 2006a,b), antioxidant effects (Lee and Jeong, 2002) and anti-metastatic activities (Lee et al., 2006). However, the physiological functions and the features of CK as an anti-allergic agent remain unclear. The objective of this study was to determine the inhibitory effect of CK on OVA-induced airway inflammation in a murine model of asthma. The parameters analyzed included: levels of Th1 and Th2 cytokines and MCP-1 chemokine in bronchoalveolar lavage (BAL) fluid, serum and BAL fluid levels of allergen-specific IgE, ROS production, leukocyte infiltration, histopathology of lung inflammation, mucus hypersecretion, lung weight, NF-κB activity, and MMP activity. Our results indicated that CK pretreatment significantly protected against OVA-induced airway inflammation in a murine model of asthma.

2. Materials and methods

2.1. Chemicals

Chicken egg ovalbumin (Grade II), Aluminum Hydroxide Gel, Giemsa solution, and Hematoxylin-Eosin Y (H&E) staining solution were obtained from Sigma Chemical Co. (St. Louis, MO). Enzyme-linked immunosorbent assay (ELISA) kits were obtained from R&D Systems (Minneapolis, MN) and BD Biosciences (San Diego, CA). Alcian blue-periodic acid Schiff (PAS) staining solution was obtained from Merck (KGaA, Darmstadt, Germany). 2′,7′-Dichlorodihydrofluorescein diacetate (DCFDA) was from Molecular Probes (Eugene, OR). All other chemicals and solvents were of the highest grade commercially available.

2.2. Preparation of CK

Aqueous extract (CK) from the root of Platycodi Radix (21 years old), supplied by Jangsaeng Doraji Co., Ltd. (Jinju, South Korea), was prepared as described previously (Lee et al., 2001): powdered root was added to distilled water (5 ml/g) and the mixture was maintained at 90 °C for 10 h, cooled to room temperature, then filtered, and lyophilized. The yield of lyophilized residue corresponded to 33.5% (33.5 g of residue for each 100 g of original dry roots). The pale-yellow extract was dissolved directly in sterilized saline. The composition of CK was described previously (Kim et al., 1995). CK was tested for the presence of contaminating LPS by the Limulus amoebocyte lysate test as described previously (Choi et al., 2001b).

2.3. Animals

Six-week-old female ICR mice were purchased from Dae Han Bio Link Co., LTD (Chungbuk, Korea). The mice were acclimatized for at least 1 week prior to use. The animals were provided Purina Rodent Chow and tap water ad libitum, and were maintained in a controlled environment at 21 ± 2 °C and 50 ± 5% relative humidity with a l2-h dark/light cycle. All animal experiments were performed according to the rules and regulations of the Animal Ethics Committee, Chosun University.

2.4. Immunization and challenge

Mice were immunized on days 1, 7, and 14 by intraperitoneal (i.p.) injection of 50 μg chicken OVA emulsified in 1 mg aluminum hydroxide adjuvant in a total volume of 100 μl of PBS. CK was dissolved in saline. Mice were intragastrically (i.g.) administered 10, 50 or 100 mg/kg/day (in 100 μl) of CK each day from days 12 to 16 consecutively. The control and OVA group were administered saline (i.g.). Animals were challenged with OVA on the final day by inhalation of 1 mg/ml OVA in PBS. The control group was immunized and challenged with PBS without drug administration (Fig. 1).

2.5. Bronchoalveolar lavage (BAL) fluid collection and leukocyte count

Each mouse was anaesthetized and the trachea was cannulated during gentle massage of the throat. BAL fluid was collected by flushing 1 ml of PBS into the lung via the trachea immediately after sacrifice; approximately 0.8 ml of BAL fluid was recovered after five lavages. The BAL fluid was centrifuged (400g, 4 °C, 5 min) and the supernatant was stored at −70 °C until measurement of cytokines. Cells from the BAL fluid were washed three times with PBS and the pellet was resuspended in 100 μl of PBS. Total cell number was counted by cytospin and a differential cell count was performed after staining with Giemsa solution. The cells were differentiated by general leukocyte morphology and 200 cells were counted in each of four random locations.

2.6. Measurement of intracellular reactive oxygen species (ROS)

The fluorescent probe, 2′,7′-dichlorofluorescein diacetate (DCFDA), was used to monitor the intracellular generation of ROS. In brief, cells from the BAL fluid were washed with PBS and then treated with 25 μM DCFDA for 20 min. Intracellular ROS were monitored using a fluorescence spectrophotometer (Varioskan, Thermo Electron Co.).

2.7. Enzyme-linked immunosorbent assay (ELISA)

The level of cytokines in BAL fluid or serum were measured by sandwich ELISA using OptEIA Set mouse IL-4, IL-5, MCP-1 and IgE kits from BD Biosciences (San Diego, CA) and DuoSet mouse TNF-α, INF-γ and IL-13 kits from R&D Systems (Minneapolis, MN) according to the manufacturer's instructions. Each concentration was calculated using a linear-regression equation obtained from the standard absorbance values.

2.8. Histopathological studies

Prior to the removal of the lung, the lung tissue and trachea were filled intratracheally with fixative (4% paraformaldehyde) using a ligature around the trachea. Lung tissue was fixed with 10% (v/v) formaldehyde. The tissues were dehydrated in

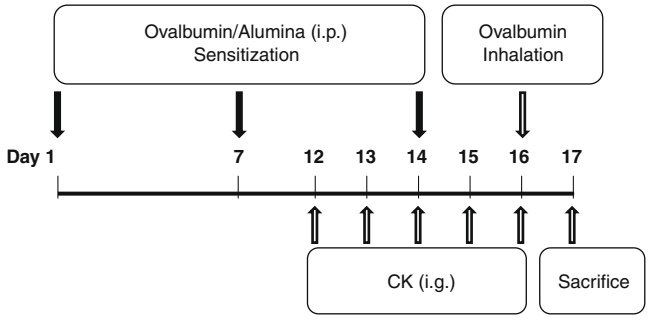

Fig. 1. Schematic diagram of the experimental protocol in mice. The mice were divided into five groups. Mice were immunized on days 1, 7, and 14 by intraperitoneal (i.p.) injection of 50 μg chicken OVA emulsified in 1 mg aluminum hydroxide adjuvant in a total volume of 100 μl of PBS. CK was dissolved in saline. Mice were intragastrically (i.g.) administered 10, 50 and 100 mg/kg/day (in 100 μl) of CK each day from days 12 to 16 consecutively. The control and OVA groups were administered saline (i.g.) without drug administration. Animals were challenged with OVA on the final day by inhalation of 1 mg/ml OVA in PBS. The control group was immunized and challenged with PBS without drug administration. Each group consisted of five mice.

various concentrations of ethanol and embedded in paraffin. For histopathological examination, 4-μm sections of fixed embedded tissues were cut on a microtome (Leica RM 2135 Rotary Microtome, Wichita, KS, United States), placed on glass slides, deparaffinized, and stained with H&E for general morphology. Airway mucus hypersecretion was evaluated using Alcian blue-PAS to stain infiltrated goblet cells. Pulmonary histopathological changes were assessed on five point scores by three independent examiners. The scores were: 0, no inflammatory cells; 1, minimal accumulation of inflammatory cells; 2, moderate accumulation of inflammatory cells; 3, severe accumulation of inflammatory cells; 4, extreme accumulation of inflammatory cells. Infiltrated goblet cells were assessed on five point scores by three independent examiners. The scores were: 0, no goblet cells; 1, minimal infiltration of goblet cells; 2, moderate infiltration of goblet cells; 3, severe infiltration of goblet cells; 4, extreme infiltration of goblet cells (Ip et al., 2006).

2.9. Western blot analysis

To obtain extracts of lung proteins, lung tissue was homogenized, washed with PBS, and incubated in lysis buffer containing a protease inhibitor cocktail (Sigma, St. Louis, MO). The samples were loaded on 10% SDS–PAGE gels and electrotransferred to nitrocellulose membrane. The blots were incubated with anti-NF-κB p65 antibody (Upstate Biotech, Lake Placid, NY) overnight at 4 °C. After washing, the blots were incubated with horseradish peroxidase-conjugated secondary antibody. Following three washes with TBST, immunoreactive bands were visualized using the ECL detection system (Pierce Biotechnology, Rockford, IL). In a parallel experiment, nuclear protein was prepared with nuclear extraction reagents (Pierce Biotechnology, Rockford, IL) according to the manufacturer's protocol.

2.10. Gelatin zymography analysis

The enzymatic activities of MMP-2 and MMP-9 were assayed by gelatin zymography in BAL fluid. BAL fluid from CK-treated mice was electrophoresed on an 8% SDS–PAGE containing 0.2% gelatin. The gel was washed twice with washing buffer (50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 2.5% Triton X-100), briefly rinsed in washing buffer without Triton X-100, and then incubated with incubation buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 10 mM $CaCl_2$, 0.02% NaN_3) at 37 °C. After incubation, the gel was stained with 0.25% Coomassie Brilliant Blue G250 (Sigma Chemical Co., St. Louis, MO) and then destained. MMP activity was represented by a clear zone of gelatin digestion.

2.11. Statistical analysis

All experiments were performed three times. Results are expressed as mean ± S.E.M. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparisons test. A significant value was defined as $P < 0.05$.

3. Results

3.1. Effects of CK on OVA-induced total and differential leukocytes in BAL fluid

We summarized the OVA-induced airway inflammation murine model of asthma in Fig. 1 and examined the change of total and differential leukocytes in the BAL fluid following OVA immunization and challenge. In the BAL fluid, the number of total cells was increased by 3.2-fold, the number of eosinophils was increased significantly by 12.6-fold, the number of basophils was increased by 3.5-fold, the number of lymphocytes was increased by 3.1-fold, and the number of macrophages was increased by 2.2-fold, compared to those in the control group. The numbers of OVA-induced eosinophils, basophils, lymphocytes, and macrophages were decreased by CK administration in a dose-dependent manner. The number of neutrophils was increased by 1.2-fold compared to that in control group, and the number of OVA-induced neutrophils was weakly inhibited by CK administration (Table 1). These results suggest that CK inhibited the OVA-induced inflammatory response in a murine model of asthma.

3.2. Effects of CK on OVA-induced histopathological changes in lung

We found that OVA-induced the infiltration of inflammatory cells into the lung tissue compared to that in the control group. CK attenuated the OVA-induced infiltration of inflammatory cells into the lung tissue (Fig. 2A). OVA also induced goblet cell hyperplasia and mucus hypersecretion into the lung tissue compared to those in the control group. CK inhibited the OVA-induced goblet cell hyperplasia and mucus hypersecretion compared to those in the OVA-inhaled group (Fig. 2B). CK also attenuated the increased inflammation and mucus score, which represents eosinophil-rich leukocyte infiltration and mucus secretion (Fig. 2C). These results suggest that CK inhibited the OVA-induced inflammatory infiltration, goblet cell hyperplasia and mucus hypersecretion in a murine model of asthma.

3.3. Effects of CK on OVA-induced levels of Th1 and Th2 cytokines in BAL fluid

We analyzed the levels of Th1 or Th2 cytokines in BAL fluid by ELISA. We found that the OVA-induced levels of cytokines were elevated compared to the control levels. The levels of TNF-α, INF-γ, IL-4, IL-5, and IL-13 were significantly increased by 4.9-fold, 1.4-fold, 4.9-fold, 6.7-fold, and 3.4-fold, respectively, compared to those in the control group. CK significantly inhibited the level of TNF-α compared to that in the OVA-induced group. The level of INF-γ was weakly inhibited by CK administration compared to that in the OVA-induced control group. The levels of IL-4, IL-5, and IL-13 were significantly inhibited by CK administration compared to those in the OVA-induced control group Table 2. In addition, the levels of Th1 and Th2 cytokines were decreased by CK administration in a dose-dependent manner. These results suggest that CK inhibited the OVA-induced levels of Th1 and Th2 cytokines in a murine model of asthma.

3.4. Effects of CK on OVA-specific IgE levels in BAL fluid and serum

We measured OVA-specific IgE levels in BAL fluid and serum by ELISA. The OVA-specific IgE levels in the BAL fluid and serum were

Table 1
Effects of CK on OVA-induced total and differential leukocytes in BAL fluid[a].

	Control	OVA	OVA + CK 10	OVA + CK 50	OVA + CK 100
Total cells (1×10^4 cells/ml)	13.5 ± 0.70	43.0 ± 2.83[b]	29.0 ± 1.42[c]	22.5 ± 0.77[c]	19.0 ± 1.41[c]
Neutrophils (1×10^4 cells/ml)	1.7 ± 0.11	2.1 ± 0.22[b]	2.0 ± 0.18	1.9 ± 0.17	1.8 ± 0.16
Eosinophils (1×10^4 cells/ml)	4.5 ± 0.70	56.5 ± 2.02[b]	26.5 ± 2.43[c]	20.5 ± 2.21[c]	12.0 ± 1.41[c]
Basophils (1×10^4 cells/ml)	6.0 ± 1.43	21.5 ± 0.77[b]	17.0 ± 1.41[c]	13.0 ± 1.44[c]	9.5 ± 0.76[c]
Lymphocytes ($1 \times t10^4$ cells/ml)	14.5 ± 0.89	44.5 ± 0.86[b]	34.0 ± 1.69[c]	27.5 ± 2.19[c]	18.0 ± 1.84[c]
Macrophages (1×10^4 cells/ml)	10.0 ± 1.58	21.5 ± 2.56[b]	20.0 ± 1.53	17.5 ± 2.65[c]	14.0 ± 1.48[c]

[a] OVA-sensitized mice were treated as described in Fig. 1. BAL fluid (0.8 ml) was collected from each animal and centrifuged. Recovered total cells were counted using cytospin. Differential leukocytes were counted using standard morphological criteria on cytospin. (cells per milliliter $\times 10^4$). At least 200 cells were examined in each cytospin. PBS-inhaled mice administered saline (Control), OVA-inhaled mice administered saline (OVA), OVA-inhaled mice administered CK 10 mg/kg/day (OVA + CK 10), OVA-inhaled mice administered CK 50 mg/kg/day (OVA + CK 50), and OVA-inhaled mice administered CK 100 mg/kg/day (OVA + CK 100). Results are the mean ± S.E.M. for five mice in each group.

[b] $P < 0.05$, significantly different from the control group.

[c] $P < 0.05$, significantly different from the OVA-treated group.

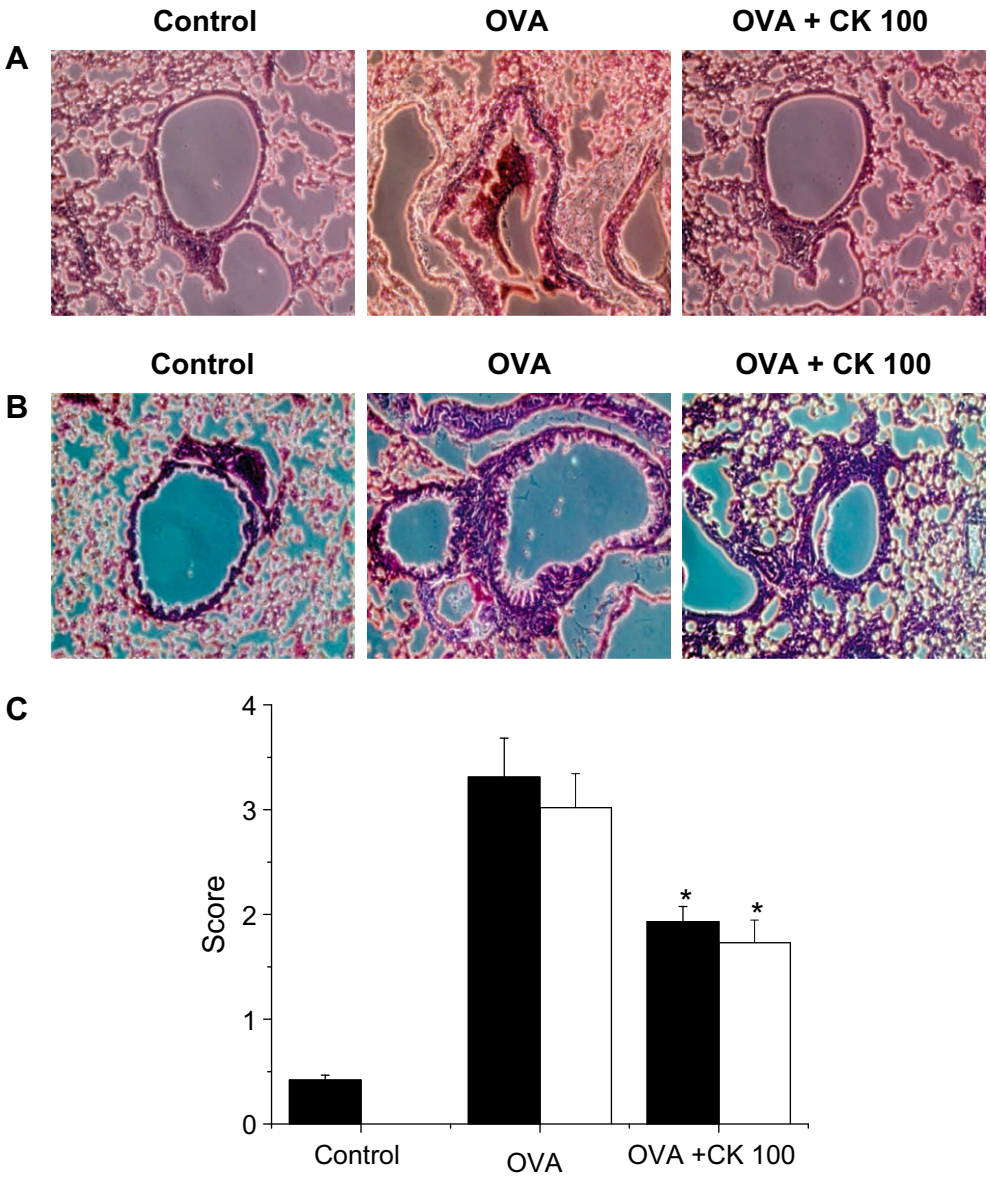

Fig. 2. Effect of CK on OVA-induced histopathological changes in lung tissue. Lung tissues were collected and fixed with 10% formaldehyde. Thin sections (4 μm) were cut and stained with (A) H&E or (B) alcian-blue-PAS. Lung tissues were obtained from PBS-inhaled mice (Control), OVA-inhaled mice (OVA), and OVA-inhaled mice administered CK (OVA + CK100), respectively. (C) Inflammatory cell infiltration (closed bar) and mucus production (open bar) in lung tissue were scored as described in the Materials and methods section. The scoring data are expressed as mean ± S.E.M. of three independent experiments. $^{*}P < 0.05$, significantly different from the OVA-inhaled group. Magnification (A and B) 200×.

Table 2
Effect of CK on OVA-induced levels of Th1 and Th2 cytokines in BAL fluid[a].

Treatment	Control	OVA	OVA + CK 10	OVA + CK 50	OVA + CK 100
TNF-α (pg/ml)	115.0 ± 13.20	574.0 ± 56.30[b]	292.0 ± 28.30[c]	218.0 ± 22.00[c]	133.0 ± 10.30[c]
INF-γ (pg/ml)	48.1 ± 4.60	66.3 ± 6.70[b]	56.9 ± 5.50	50.4 ± 4.70[c]	48.0 ± 4.50[c]
IL-4 (pg/ml)	6.7 ± 0.70	33.4 ± 3.50[b]	25.9 ± 2.20[c]	21.7 ± 2.30[c]	15.0 ± 1.50[c]
IL-5 (pg/ml)	78.0 ± 8.30	527.0 ± 53.20[b]	328.0 ± 33.20[c]	232.0 ± 26.10[c]	135.0 ± 14.70[c]
IL-13 (pg/ml)	215.0 ± 23.40	730.0 ± 69.40[b]	527.0 ± 53.10[c]	435.0 ± 34.50[c]	295.0 ± 20.10[c]

[a] OVA-sensitized mice were treated as described in Fig. 1. BAL fluid was collected 24 h after the last airway challenge. The levels of cytokines in BAL fluid were measured using ELISA Kits. PBS-inhaled mice administered saline (Control), OVA-inhaled mice administered saline (OVA), OVA-inhaled mice administered CK 10 mg/kg/day (OVA + CK 10), OVA-inhaled mice administered CK 50 mg/kg/day (OVA + CK 50), and OVA-inhaled mice administered CK 100 mg/kg/day (OVA + CK 100). Results are the mean ± S.E.M. for five mice in each group.

[b] $P < 0.05$, significantly different from the control group.

[c] $P < 0.05$, significantly different from the OVA-treated group.

significantly increased by 10.7-fold and by 10.8-fold, respectively, compared to the control group. The OVA-specific IgE level was significantly inhibited by CK administration in a dose-dependent manner (Fig. 3A and B). These results suggest that CK inhibited the allergen- or OVA-specific IgE level in a murine model of asthma.

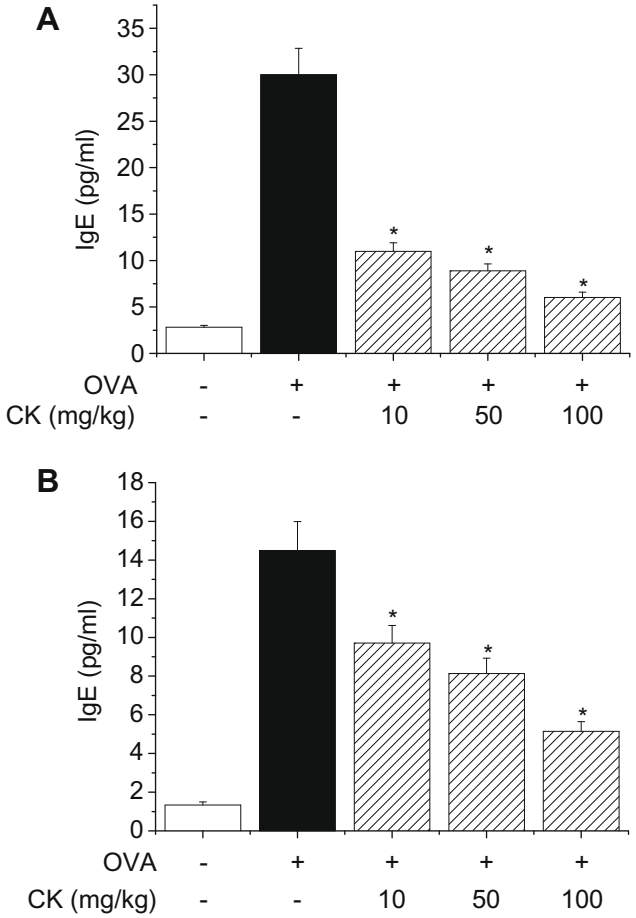

Fig. 3. Effect of CK on OVA-specific IgE levels in BAL fluid and serum. BAL fluid was collected from OVA-induced mice treated with or without 10, 50 and 100 mg/kg of CK between the sensitization and inhalation period. The control group was sensitized and challenged with PBS without drug administration. The OVA-specific IgE levels in BAL fluid (A) and serum (B) were analyzed by ELISA as described in the Materials and methods section. Results are expressed as the mean ± S.E.M. of three independent experiments. $^{*}P < 0.05$, significantly different from the OVA-inhaled group.

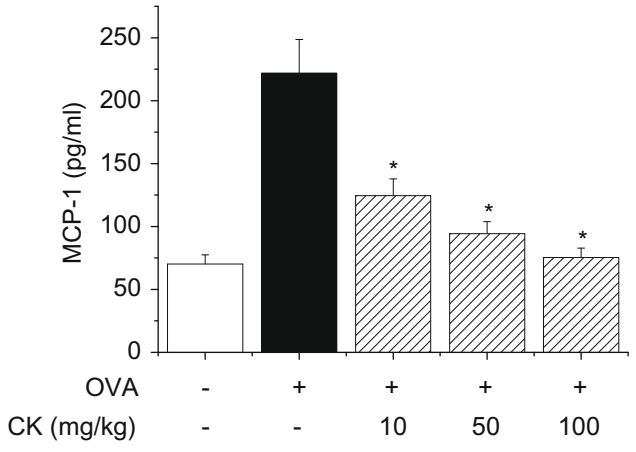

Fig. 4. Effect of CK on OVA-induced level of MCP-1 chemokine in BAL fluid. BAL fluid was collected from OVA-induced mice treated with or without 10, 50 and 100 mg/kg of CK between the sensitization and inhalation period. The control group was sensitized and challenged with PBS without drug administration. The levels of MCP-1 chemokine in BAL fluid were analyzed by ELISA as described in the Materials and methods section. Results are expressed as the mean ± S.E.M. of three independent experiments. $^{*}P < 0.05$, significantly different from the OVA-inhaled group.

3.5. Effects of CK on the OVA-induced level of MCP-1 chemokine in BAL fluid

We measured the level of MCP-1 chemokine in BAL fluid by ELISA. The OVA-induced level of MCP-1 chemokine was significantly increased by 3.2-fold compared to the level in control. CK significantly inhibited the level of MCP-1 compared to that in the OVA-induced group (Fig. 4). These results suggest that CK inhibited the OVA-induced level of MCP-1 chemokine in a murine model of asthma.

3.6. Effects of CK on OVA-induced lung weight

We examined the OVA-induced airway hypertrophy in the lung tissue by assessing histological changes compared to control lung tissue (Fig. 2). Lung weight in the OVA-induced group was slightly increased by 1.5-fold compared to that in the control group. Total lung weight was weakly inhibited by CK administration compared to that in the OVA-induced group (Fig. 5). These results suggest that CK inhibited the OVA-induced total lung weight gain in a murine model of asthma.

3.7. Effects of CK on OVA-induced ROS generation in BAL fluid

Oxidative stress occurs in many allergic and immunologic disorders. Many studies have shown increased production of ROS in asthma, allergic rhinitis, and atopy dermatitis that contributes, in part, to tissue injury at sites of inflammation (Bowler and Crapom, 2002). We examined the effect of CK on ROS generation, as described in Fig. 6. For this experiment, samples were collected at 24 h after the last challenge. The level of ROS in BAL fluid was increased significantly at 24 h after inhalation of OVA compared to the levels after inhalation of PBS. ROS generation was decreased by CK administration compared to that in the OVA-induced group (Fig. 6). These results suggest that CK exerted an inhibitory effect on ROS generation.

3.8. Effects of CK on NF-kB nuclear translocation in OVA-induced lung

Many studies have demonstrated that NF-κB activation is involved in the chronic feature of airway inflammation in asthma. We investigated the effect of CK on NF-κB p65 nuclear translocation in a murine model. The lung tissues were collected from control and CK-treated mice 2 h after the final airway OVA challenge,

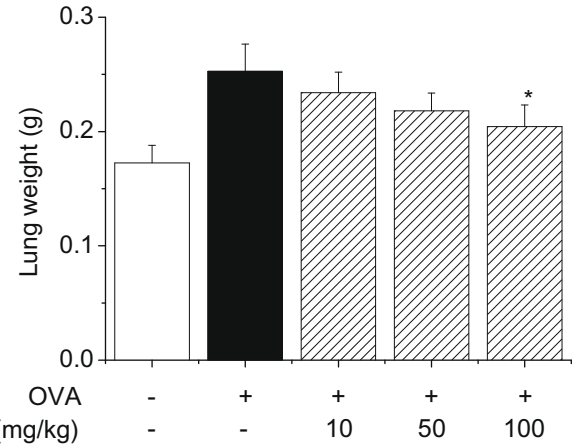

Fig. 5. Effect of CK on OVA-induced lung weight. Lung tissues were collected and washed with PBS. The OVA-induced lung weight was measured by portable balance (Voyager® Pro, OHAUS). Results are expressed as the mean ± S.E.M. of three independent experiments. $^{*}P < 0.05$, significantly different from the OVA-inhaled group.

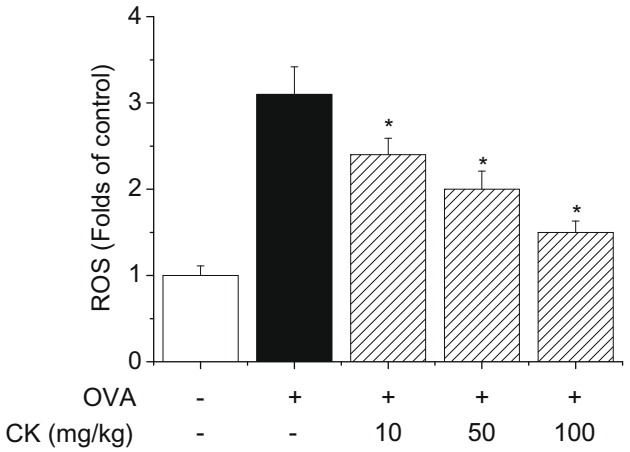

Fig. 6. Effects of CK on OVA-induced ROS generation in BAL fluid. Cells in the BAL fluid were washed with PBS. Cells were treated with 25 μM DCFDA for 20 min. Intracellular ROS were monitored using a fluorescence spectrophotometer (Varioskan, Thermo Electron Co.). Results are expressed as the mean ± S.E.M. of three independent experiments. $^{*}P < 0.05$, significantly different from the OVA-inhaled group.

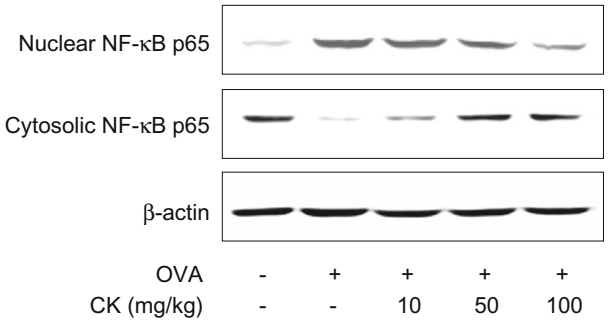

Fig. 7. Effects of CK on NF-κB nuclear translocation in OVA-induced lung tissue. NF-κB p65 protein expression in nuclear and cytosolic protein extracts from lung tissue. NF-κB p65 protein expression was measured 2 h after the last challenge in PBS-inhaled mice administered saline, OVA-inhaled mice administered saline, and OVA-inhaled mice administered CK. Results were similar in three independent experiments.

and cytosolic and nuclear proteins were extracted as described in Section 2. The increased levels of NF-κB p65 in the nuclear protein extracts from lung tissue at 2 h after OVA inhalation were decreased by CK administration. In contrast, levels of NF-κB p65 protein in the cytosolic protein extracts from lung tissue were decreased at 2 h after OVA inhalation compared to the levels in control group (Fig. 7). The decreased levels of NF-κB p65 in the cytosolic protein extracts from lung tissue at 2 h after OVA inhalation were increased by CK. These results suggest that CK inhibited NF-κB activity by preventing translocation of this transcription factor into the nucleus.

3.9. Effects of CK on MMPs activities in BAL fluid

Many studies have demonstrated that MMPs are involved in the proteolytic degradation of environmental barriers and are up-regulated in regions of lung tissue remodeling (Kumagai et al., 1999). We assessed the activities of MMP-2 and -9 in BAL fluids by gelatin zymography. OVA significantly increased the activities of MMP-2 and -9 compared to those in the control, and CK significantly decreased the activities of MMP-2 and -9 compared to the OVA-induced group (Fig. 8). These results suggest that CK inhibited the OVA-induced activities of MMP-2 and -9 in a murine model of asthma.

4. Discussion

Changkil (CK), which is the aqueous extract from the root of Platycodi Radix cultivated for more than 20 years, has anti-inflammatory effects (Kim et al., 2006a,b; Ahn et al., 2005). Nevertheless, the effect of CK on airway inflammation remains largely unknown. OVA-induced asthma is a disease that results from chronic airway inflammation characteristically associated with the infiltration of macrophages, lymphocytes, and eosinophils into the bronchial lumen (Selgrade et al., 2008; Roh et al., 2008). In this study, we determined that CK significantly reduced the characteristics of airway inflammation, including AHR, infiltration of inflammatory cells, and production of inflammatory cytokines. In addition, CK decreased the activity of NF-κB and ROS in the OVA-induced airway inflammation reaction.

Inflammatory cells recruited to asthmatic airways have an exceptional capability to produce ROS. At sites of inflammation, multiple inflammatory cells, including eosinophils, neutrophils, and macrophages, are capable of generating ROS, which can participate in the development of a variety of diseases, including allergic asthma (Conner and Grisham, 1996; Leusen et al., 1996; Babior, 1999). Such ROS may contribute to tissue injury and inflammatory reactions. Consistent with these findings, our present results showed that ROS generation in BAL fluid, which mainly consists of recruited inflammatory cells, was significantly increased in the OVA-induced group. The increased ROS generation was substantially reduced by CK.

Previous studies have shown that several proteins, including various transcriptional factors, are involved in ROS-based regulation of signal transduction and gene expression in various immune and inflammatory processes (Sen, 1998; Lee et al., 2005). ROS promote the activities of proinflammatory redox-sensitive nuclear factors, including NF-κB, thus increasing the occurrence of allergic inflammation in asthmatic patients (Rahman, 2003; Henderson et al., 2002). NF-κB is detected in most cell types and has a critical function in immune and inflammatory responses, including asth-

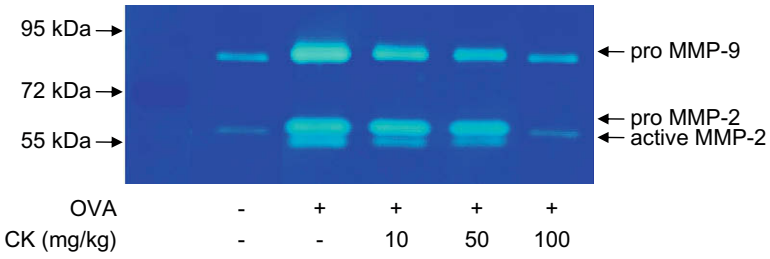

Fig. 8. Effect of CK on the gelatinolytic activities of MMPs in the BAL fluids of OVA-induced asthmatic mice. MMPs in BAL fluids collected from mice as described in Materials and methods section were analyzed by gelatin zymography. Results were similar in three independent experiments.

ma (Barnes and Karin, 1997; Siebenlist et al., 1994; Zhou et al., 2006; Baldwin, 1996). As expected, the NF-κB protein level was substantially increased in the OVA-induced model of asthma used in the present study. Activation of this transcription factor induces a variety of inflammatory genes that are abnormally expressed in asthma. These genes include cytokines (e.g., IL-4, IL-5, IL-13, TNF-α and INF-γ) and chemokines (e.g., eotaxin and MCP-1) (Barnes and Karin, 1997; Stütz and Woisetschläger, 1999; Chvatchko et al., 2003; Mori et al., 1999). In this study, we found that CK effectively suppressed NF-κB activity in the lung tissue via a reduction in oxidative stress. Therefore, inhibitors of NF-κB are effective compounds for producing anti-inflammatory effects in general and also in asthma.

The levels of TNF-α, IL-4, IL-5, IL-13, IgE, and MCP-1 were significantly elevated by airway challenge with OVA, but the levels of Th1 and Th2 cytokines, IgE, and MCP-1 were significantly inhibited after CK administration compared to those in the OVA-induced group. The level of IFN-γ was slightly elevated by airway challenge with OVA, and IFN-γ level was weakly decreased in the CK-treated group compared to the OVA-induced group. Histopathological investigation has shown increased submucosal and adventitial deposition of matrix proteins that comprise fibronectin and collagens I, III and V as well as an increase in smooth muscle that account for much of the airway wall thickening (Holgate, 2008). Airway wall remodeling accompanies a decline in lung function that has been observed in severe asthma. The asthmatic lung tissue, the infiltrated inflammatory cells or the mucus generation of goblet cells was markedly induced by airway challenge with OVA, but the induction of infiltrated inflammatory cells or mucus generation of goblet cells was markedly attenuated by CK administration. Airway wall remodeling is characterized by goblet cell hyperplasia and the deposition of collagens (Wills-Karp and Karp, 2004), and MMPs are expressed in the airway during periods of airway remodeling. CK significantly decreased the activities of MMP-2 and -9 compared to those in the OVA-induced group.

In conclusion, this study is the first to provide experimental evidence demonstrating that CK inhibits OVA-induced airway inflammation in a murine model of asthma. Administration of CK significantly inhibited asthmatic reactions such as leukocyte recruitment and the levels of Th1 and Th2 cytokines, IgE, and MCP-1 chemokine in the lung. Furthermore, CK decreased ROS generation, NF-κB translocation, and MMP activities in the OVA-induced airway inflammation reaction. Our results suggest that CK is an excellent candidate as an adjuvant therapy for bronchial asthma patients.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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