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Curcumin attenuates allergic airway inflammation and hyper-responsiveness in mice through NF-κB inhibition

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ABSTRACT

Ethnopharmacological relevance: Curcumin, a polyphenol compound from *Curcuma longa* L. has been used for centuries as an anti-inflammatory remedy including asthma. Curcumin has been reported to exert an anti-inflammatory effect, in part, through inhibition of the NF-κB pathway.
Aim of the study: The purposes of this study were to determine whether curcumin inhibits NF-κB-dependent transcription *in vitro*, and test whether treatment with curcumin reduces allergen-induced airway inflammation and hyper-responsiveness in a mouse model of asthma through inhibition of NF-κB pathway.
Materials and methods: The effect of curcumin on NF-κB transcriptional activity was investigated using a cell-based luciferase reporter assay in A549 cells and by measuring inhibitory κBα (IκBα), p65, and p50 levels after exposure of Raw264.7 cells to lipopolysaccharide (LPS). BALB/c mice were sensitized to ovalbumin (OVA) by intraperitoneal injection, and challenged with repeated exposure to aerosolized OVA. The effects of daily administered curcumin (200 mg/kg body weight, i.p.) on airway hyper-responsiveness (AHR), inflammatory cell number, and IgE levels in bronchoalveolar lavage (BAL) fluid were analyzed. NF-κB activation in lung tissue was also assessed by Western blot analyses.
Results: Curcumin inhibited NF-κB-dependent transcription in reporter assays in A549 cells with an IC_{50} of 21.50 ± 1.25 μM. Curcumin stabilized IκBα and inhibited nuclear translocation of p65 and p50 in LPS-activated Raw264.7 cells, and curcumin-treated mice showed reduced nuclear translocation of p65 in lung tissue. Treatment with curcumin significantly attenuated AHR and reduced the numbers of total leukocytes and eosinophils in BAL fluid. Infiltration of inflammatory cells and mucus occlusions in lung tissue were significantly ameliorated by treatment with curcumin, which also markedly decreased the level of IgE in BAL fluid.
Conclusion: Curcumin attenuates the development of allergic airway inflammation and hyper-responsiveness, possibly through inhibition of NF-κB activation in the asthmatic lung tissue. Our results indicate that curcumin may attenuate development of asthma by inhibition of NF-κB activation.

1. Introduction

Curcuma longa L., which belongs to the Zingiberaceae family, is a perennial herb that measures up to 1 m high with a short stem, distributed throughout tropical and subtropical regions of the world, being widely cultivated in Asiatic countries, mainly in India and China. Curcumin is a polyphenol present in the rhizome of the plant *Curcuma longa* Linn. Curcumin, in the form of the herbal powder, turmeric (given the name curry spice by the British), has been used for centuries as an anti-inflammatory remedy in Asian medicine (Srinivasan, 1953). Turmeric has been widely consumed traditionally for gastrointestinal and respiratory problems such as chronic diarrhea, flatulence, cough, cold, asthma, and throat irritations (Gilani et al., 2005).

Asthma is an inflammatory disease of the airways, characterized by lung eosinophilia, mucus hypersecretion by goblet cells, and airway hyper-responsiveness (AHR) to inhaled allergens (Elias et al., 2003). Several transcription factors have been reported to be involved in the inflammatory process in asthma, including the glucocorticoid receptor (GR), nuclear factor-κB (NF-κB), activator protein-1 (AP-1), nuclear factor of activated T-cells (NF-AT), and cyclic AMP response element binding protein. More recent

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studies have implicated CCAAT/enhancer binding protein (C/EBP); peroxisome proliferator-activated receptor (PPAR); and the bZIP transcription factor, Nrf2 (Roth and Black, 2006).

Asthma development is associated with the expression of a broad array of NF-κB-regulated inflammatory proteins, including cytokines, enzymes, and adhesion molecules. Several lines of evidence suggest a central role for NF-κB in the pathogenesis of asthma: Activated NF-κB has been identified in key locations in the airways of asthmatic patients. Agents known to exacerbate asthma, such as allergens, ozone, and viral infections, stimulate activation of NF-κB. Corticosteroid, the major effective treatment for asthma, is a potent inhibitor of NF-κB activation. Certain NF-κB-dependent chemokines, such as regulated upon activation normal T-cell expressed and secreted (RANTES) and eotaxin, function to recruit eosinophils in the airway—a typical feature of asthma (Christman et al., 2000; Wright and Christman, 2003).

The anti-inflammatory effect of curcumin is associated with inhibition of cyclooxygenase, lipoxygenase, and prostaglandin synthesis (Kapoor, 1990). Curcumin has been shown to inhibit lipopolysaccharide (LPS)-induced activation of NF-κB and reduce the biological activity of tumor necrosis factor (TNF) in L929 fibroblast lytic assays (Huang et al., 1991). These results indicate that curcumin may have a beneficial effect in asthma through inhibition of the NF-κB pathway. In this study, we examined the effect of curcumin in ovalbumin (OVA)-induced airway inflammation in a mouse model of asthma.

2. Materials and methods

2.1. NF-κB luciferase reporter assay

The activity of curcumin toward NF-κB-dependent transcription was tested in A549 (human alveolar epithelial cancer) cells stably transfected with an NF-κB-responsive luciferase reporter construct. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1× penicillin/streptomycin (10,000 units/ml penicillin and 10,000 μg/ml streptomycin in 0.85% NaCl), and 250 mg/ml G418. For assays, cells were harvested with Hank's Balanced Salt Solution (HBSS) containing 0.05% trypsin and 0.53 mM EDTA, diluted with assay medium (same as above except lacking G418), plated in a 96-well plate at 2×10^4 cells/well in a total volume of 50 μl/well, and incubated at 37 °C in a CO_2 incubator for 24 h. Phorbol myristate acetate (PMA; final concentration, 10 ng/ml) and curcumin in 0.05% DMSO or a 0.05% DMSO solution (vehicle control) were then added to each well. Bright-Glo solution (100 μl/well; Promega, Madison, WI, USA) was added to each well after incubating for 6 h at 37 °C in a CO_2 incubator, and luciferase activity was measured with a luminometer (MicroLumat, Berthold, Germany). Each assay was performed in duplicate.

2.2. Raw264.7 cells

The murine macrophage-like Raw264.7 cell line was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in DMEM with 10% FBS. Raw264.7 cells were exposed to 0.1 μg/ml LPS (011:B4) for 30 min and harvested with HBSS containing 0.05% trypsin and 0.53 mM EDTA for Western blot analysis. Raw264.7 cells were preincubated for 4 h with the indicated.

2.3. Mice

The specific pathogen-free (SPF), female BALB/c mice (6–8 weeks of age) were purchased from Orientbio Inc. (Seongnam, Korea). All animal study protocols were approved by the Animal Care Committee of Seoul National University. The mice were divided into four groups: (1) alum-sensitized/non-challenged + saline-treated (SAL); (2) OVA-sensitized/challenged + vehicle treated (OVA); (3) OVA-sensitized/challenged + curcumin-treated mice by oral gavage (CMN-PO); and (4) OVA-sensitized/challenged + curcumin-treated mice by intraperitoneal (i.p.) injection (CMN-IP).

2.4. Allergen sensitization/challenge protocol

Mice underwent OVA-sensitization and challenge as previously described with slight modifications (Oh et al., 2002). OVA (500 μg/ml; Pierce, Rockford, IL, USA) in PBS was mixed with an equal volume of 10% (w/v) aqueous aluminum potassium sulfate (alum; Sigma, St Louis, MO, USA) and incubated for 60 min at room temperature after adjusting the pH to 6.5 using 10 N NaOH. After centrifugation at 750 × *g* for 5 min, the OVA/alum pellet was resuspended at its original volume in distilled water. The mice received an intraperitoneal injection of 100 μg OVA (0.2 ml of 500 μg/ml solution in normal saline) complexed with alum on the first day, and were challenged intranasally (i.n.) on days 8, 15, 18, and 21, as described by Henderson et al. (Oh et al., 2002). Mice were prepared for challenge by anesthetizing with an intraperitoneal (i.p.) injection of 0.2 ml of a mixture of ketamine (0.44 mg/ml) and xylazine (6.3 mg/ml) in normal saline, and were placed on a board in the supine position. On day 8, mice were challenged with 250 μg OVA (100 μl of a 2.5 mg/ml solution); on days 15, 18, and 21, mice were challenged with 125 μg OVA (50 μl of 2.5 mg/ml solution). For the Western blot analysis of NF-κB activation status, a separate group of mice was sensitized and challenged on days 8 and 15. Mice were euthanized and lung samples were collected 1 and 4 h after OVA challenge on day 15. The pattern of OVA deposition achieved by i.n. delivery was examined by i.n. administration of 100 μl of a mixture of OVA (2.5 mg/ml) in toluidine blue. The majority of toluidine blue dye staining was seen in the lumen and interstitium of the tracheal wall; the remainder was detected in the lumen and interstitium of the small airways. Toluidine blue dye staining was not detected in the esophagus or stomach. The control mice received an i.p. injection of saline with alum on the first day and an i.n. administration of saline on days 8, 15, 18, and 21.

2.5. Treatment with curcumin

Curcumin (Sigma, St. Louis, MO, USA), at a concentration of 20 mg/ml, was suspended in saline containing 0.5% carboxymethylcellulose (CMC) and given by oral gavage (CMN-PO) or by i.p. injection (CMN-IP) at a dose of 200 mg/kg body weight once daily from day 1 to day 21; the SAL and OVA groups received saline containing 0.5% CMC. Satellite number of mice for investigation of NF-κB activation status received curcumin or saline from day 1 to day 15.

2.6. AHR

In vivo airway responsiveness to methacholine was measured 24 h after the last OVA challenge in conscious, freely moving, spontaneously breathing mice, using whole-body plethysmography (Model PLY 3211; Buxco Electronics, Sharon, CT, USA). The mice were challenged with aerosolized saline or methacholine (3, 10, and 30 mg/ml), generated by an ultrasonic nebulizer (DeVilbiss Health Care, Somerset, PA, USA) for 1 min. The degree of bronchoconstriction was expressed as enhanced pause (P_{enh}), a calculated dimensionless value that correlates with measurements of airway resistance, impedance, and intrapleural pressure in the

same mouse. P_{enh} values, based on readings collected over 4 min and averaged after each nebulization challenge, were calculated as follows: $P_{enh} = [(T_e/T_r - 1) \times (PEF/PIF)]$, where T_e is the expiration time, T_r is the relaxation time, PEF is the peak expiratory flow, and PIF is the peak inspiratory flow × 0.67 (a coefficient). The time for the box pressure to change from a maximum to a user-defined percentage of the maximum represents the relaxation time. The T_r measurement was begun at the maximum box pressure and ended at 40%.

2.7. Bronchoalveolar lavage

After measurement of AHR, the mice were exsanguinated by cardiac puncture, and a bronchoalveolar lavage (BAL) was performed on the right lung (0.4 ml saline, three times) after tying off the left lung at the main stem bronchus. Total BAL fluid cells in a 0.05-ml aliquot were counted, and the remaining fluid was centrifuged at 200 × *g* and 4 °C for 10 min. The cell pellets were resuspended in saline containing 10% BSA, and prepared as smears on glass slides. Eosinophils were stained for 5 min with 0.05% aqueous eosin and 5% acetone in distilled water, rinsed with distilled water, and counterstained with 0.07% methylene blue.

2.8. IgE level in BAL fluid

Levels of IgE in BAL fluid were determined by ELISA, according to the manufacturer's protocols (R&D Systems, Minneapolis, MN, USA). Lower limit of the quantification of this assay was 10 ng/ml.

2.9. Histopathology

After BAL, the trachea and upper and lower lobes of the left lung were removed, and fixed for 24 h in 10% neutral buffered formalin solution. The tissues were embedded in paraffin, cut into 3-μm sections, stained with hematoxylin and eosin (H&E) or Alcian Blue at pH 2.5, and counterstained with Nuclear Fast Red to identify airway goblet cells and mucus. The degree of airway inflammatory cell infiltration, mucus occlusion of the airway diameter, and airway edema were determined by morphometry. Morphometric analyses were performed by individuals blinded to the protocol design, as previously described with minor modification (Choi et al., 2005). Briefly, for each mouse, eight airway sections, randomly distributed throughout the left lung, were analyzed, and the average score of each point was calculated. The occlusion of the airway diameter by mucus was assessed on a semiquantitative 0–4+ scale based on the following criteria: 0, no mucus; 1, ~25% occlusion, 2, ~50% occlusion; 3, ~75% occlusion; 4, ~100% occlusion. The degree of inflammatory cell infiltration around bronchioles, peribronchial arteries, and veins was scored using the following criteria: 0, no infiltration; 1, ~3 layers of inflammatory cells; 2, ~6 layers of inflammatory cells; 3, ~10 layers of inflammatory cells; 4, more than 10 layers of inflammatory cells. The degree of edema was assessed according to the following criteria: 0, no edema; 1, ~25% airway involved; 2, ~50% airway involved; 3, ~75% airway involved; 4, ~100% airway involved.

2.10. Western blot analysis

Nuclear and total proteins in Raw264.7 cells and lung tissue were prepared using NE-PER nuclear and cytoplasmic extraction reagents (Pierce Biotechnology, Rockford, IL, USA) according to the manufacturer's protocol. Equal amounts of protein extracts were separated by SDS-PAGE on 10% gels and then electrotransferred to nitrocellulose membranes. The blots were incubated with an anti-NF-κB p65 antibody (Cell Signaling Technology, Lexington, KY, USA) overnight at 4 °C. After washing, the blots were incubated with a horseradish peroxidase-conjugated secondary antibody. Following three washes with Tris-buffered saline (pH 7.5) containing 0.05% Tween-20 (TBST), immunoreactive bands were visualized by enhanced chemiluminescence detection (ECL; Pierce Biotechnology, Rockford, IL, USA). Equal loading was assessed by staining membranes with Ponceau dye (Sigma, St. Louis, MO, USA) and then reprobing with an anti-GADPH antibody (Cell Signaling Technology, Danvers, MA, USA). For the quantification of expression levels, the blots were scanned and the density of each band was determined using image analysis software (Bio-1D Version 99.03, Vilber Lourmat, Eberhardzell, Germany) and normalized against the density of the GADPH band.

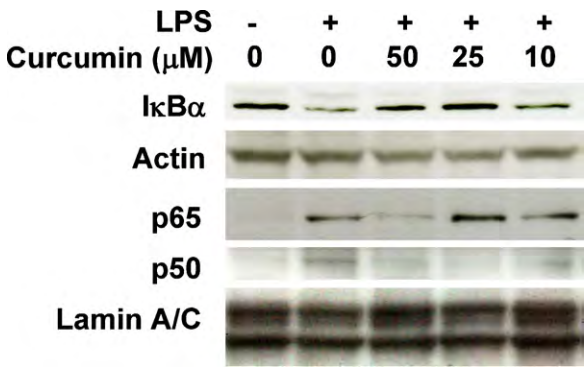

Fig. 1. Effect of curcumin on LPS-induced IκBα degradation and p65 and p50 nuclear translocation in Raw264.7 cells. Raw264.7 cells were treated with curcumin for 4 h prior to a 30-min exposure to LPS. Western blot analyses were performed using the cytoplasmic fraction (IκBα and actin) and nuclear fraction (p65, p50, and Lamin A/C) of cell lysates.

2.11. Hematological examination

Whole blood was collected at autopsy (day 22) from the vena cava with a heparinized syringe. The number of white blood cells (WBCs) and red blood cells (RBCs), and hemoglobin content were measured with an automatic hematology analyzer (Coulter JT, Beckman Coulter, CA, USA).

2.12. Statistical analyses

The data are reported as means ± standard errors of the means (SEMs). Differences between groups were analyzed by one-way analysis of variance (ANOVA); p-values < 0.05 were considered significant. The pathological grades of each group were compared using the non-parametric Mann–Whitney test using SigmaStat software (Version2.0, SPSS Inc., CA, USA).

3. Results

3.1. Effect of curcumin on NF-κB transcriptional activity

PMA induced an increase in NF-κB-dependent transcriptional activity in A549 cells that was dose-dependently inhibited by curcumin with an IC_{50} of 21.5 ± 1.25 μM.

3.2. Effect of curcumin on NF-κB activation in Raw264.7 cells

LPS activated the NF-κB pathway in Raw264.7 cells, as shown by a decrease in the levels of cytoplasmic IκBα and an increase in p65 and p50 nuclear translocation. Curcumin stabilized the IκBα and inhibited nuclear translocation of p50 at 25 and 50 μM. Inhibition of nuclear translocation of p65 was evident at 50 μM (Fig. 1). Cur-

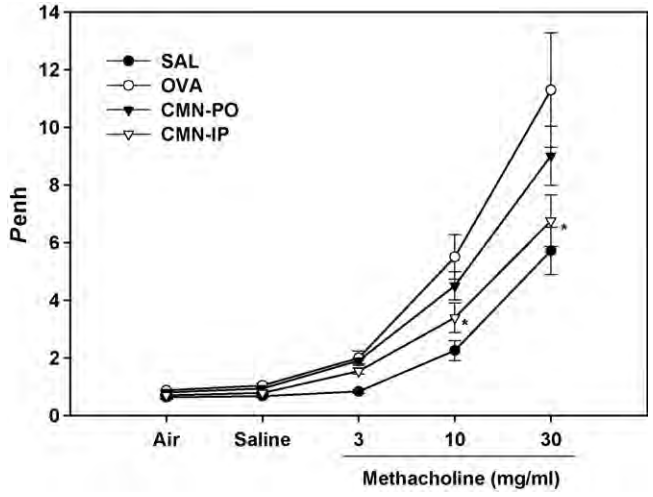

Fig. 2. Effect of curcumin on AHR to methacholine in OVA-treated mice. The degree of bronchoconstriction, expressed as P_{enh}, to inhaled methacholine (3, 10, and 30 mg/ml) was determined in saline-treated (SAL; $n = 6$), OVA control (OVA; $n = 8$), curcumin-treated by oral gavage (CMN-PO; $n = 8$), and curcumin-treated by i.p. injection (CMN-IP; $n = 8$) mice.

cumin did not modulate the cytoplasmic level of IκBα nor nuclear translocation of p65 and p50 at 10 μM.

3.3. Effect of curcumin on AHR

AHR to inhaled methacholine at 10 and 30 mg/ml was significantly increased in the OVA group compared with that in saline-treated mice ($p < 0.05$). Treatment with curcumin by i.p. injection significantly reduced AHR at 3 mg/ml ($p < 0.01$), 10 mg/ml ($p < 0.01$), and 30 mg/ml ($p < 0.05$) methacholine compared with that in the OVA control group. Treatment of curcumin by oral gavage reduced AHR at 10 mg/ml and 30 mg/ml methacholine, but it was not significant (Fig. 2).

3.4. Effect of curcumin on allergic airway inflammation

OVA challenge in OVA-sensitized mice caused a marked infiltration of leukocytes into the lung, increasing the total number of leukocytes recovered in BAL fluid by 4.3-fold compared with that in the saline-treated group (Fig. 3A). In the OVA group, 59.9% of the total leukocytes in BAL fluid were eosinophils, whereas only 1.0% of BAL fluid leukocytes were eosinophils in the saline-treated group. Treatment with curcumin by i.p. injection and oral gavage decreased the number of BAL fluid leukocytes by 50% and 43% compared with the OVA control group ($p < 0.05$ and $p = 0.07$) (Fig. 3A), and also reduced the number of eosinophils by 54% and 35% respectively ($p < 0.05$ and $p = 0.07$) (Fig. 3B). Light microscopy revealed that the lungs of saline-treated mice were normal in appearance without inflammatory cells in the airways, whereas the OVA group showed a marked infiltration of inflammatory cells, including eosinophils and mononuclear cells, in peribronchial and perivascular spaces and alveolar walls. Curcumin treatment by i.p. injection markedly reduced the inflammatory changes in OVA-sensitized/challenged mice (Figs. 4 and 5A). Airway edema and mucus occlusions were also evident in OVA-treated mice. OVA-induced mucus occlusions were significantly reduced by curcumin treatment by i.p. injection ($p < 0.05$) (Fig. 5B), and there was also a trend toward reduced edema in the curcumin-treated with i.p. injection group (Fig. 5C), although this difference did not reach statistical significance ($p = 0.17$). Curcumin treatment by oral gavage reduced inflammatory cell infiltration, mucus occlusion and edema.

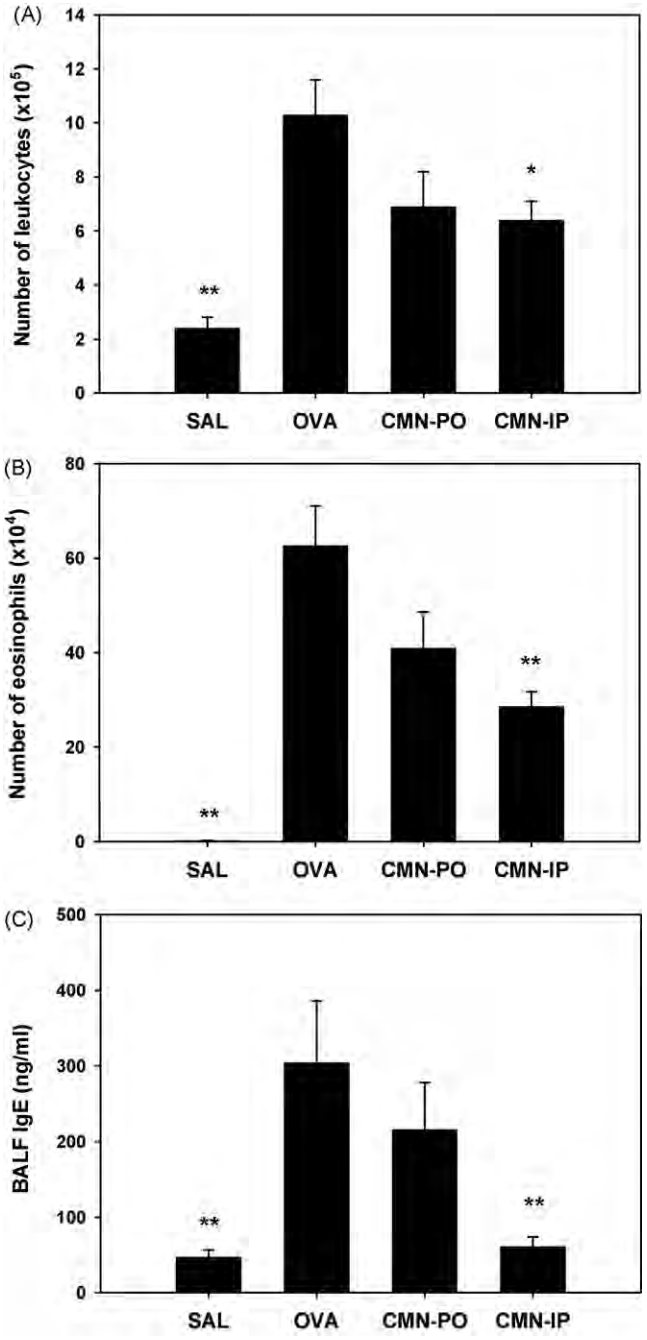

Fig. 3. Effect of curcumin on the number of inflammatory cells and IgE level in BAL fluid. BAL fluid was obtained from saline-treated ($n = 6$), OVA control ($n = 10$), OVA + CMN-treated by oral gavage (CMN-PO, $n = 10$) and OVA + CMN-treated by i.p. injection (CMN-IP, $n = 10$) mice. The numbers of total leukocytes (A) and eosinophils (B), and the level of total IgE (C) present in the BAL fluid from each group were determined. The results shown are the means ± SEMs. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the OVA control group.

However, all these decrease were not significantly different from OVA control group.

3.5. Effect of curcumin on IgE release

IgE levels in BAL fluid were markedly increased in OVA-treated mice compared with saline-treated mice (47 ± 9.4 ng/ml vs 333.5 ± 60.5 ng/ml; $p < 0.01$). As shown in Fig. 3C, treatment with curcumin either by oral gavage or i.p. injection reduced IgE levels by 34% and 95% compared with the OVA control group.

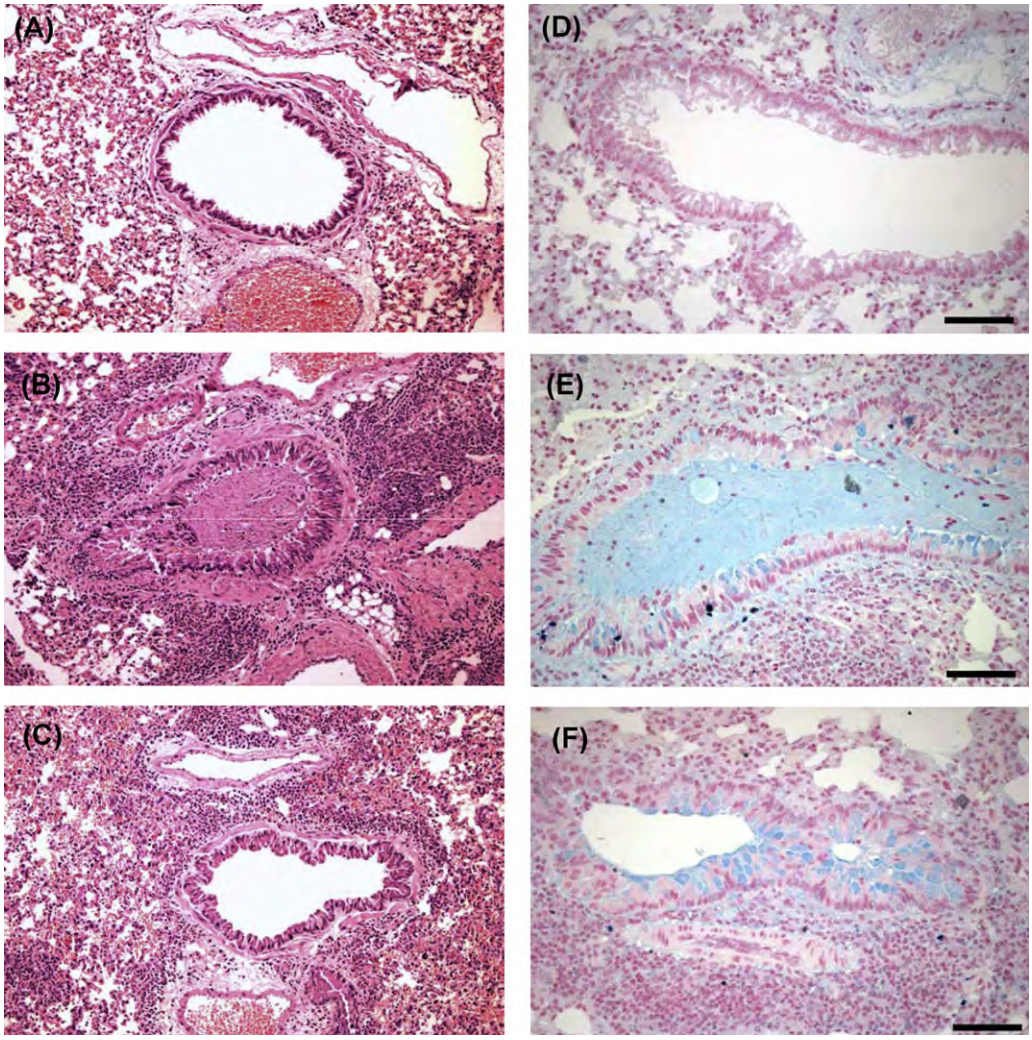

Fig. 4. Qualitative effects of curcumin on airway inflammation (images). Representative images of lung sections from saline-treated (A, D), OVA control (B, E), and OVA + CMN-treated by i.p. injection (C, F) groups are shown. The lung sections were stained with H&E (A–C) or Alcian Blue with Nuclear Fast Red counterstaining (D–F), and examined by light microscopy.

3.6. Effect of curcumin on NF-κB activation in asthmatic lung tissue

Nuclear translocation of p65 was significantly increased 1 and 4 h after OVA challenge in OVA-treated mice compared with saline-treated mice ($p < 0.05$ for all), but was not different 24 h after the last OVA challenge on day 21 (data not shown). The level of p65 expression in the nuclear fraction of tissue lysates was reduced in curcumin-treated (i.p.) mice compared with OVA-treated mice at 4 h after OVA challenge. Conversely, the level of p65 expression in the cytosolic faction was increased in curcumin-treated mice compared with OVA-treated mice 1 and 4 h after OVA challenge (Fig. 6).

3.7. Toxicity

Possible side effects of curcumin were monitored by measuring body weights and evaluating mice for clinical evidence of toxicity; no differences were found between groups. The body weights of saline-treated (22.7 ± 1.0 g), OVA control (20.3 ± 0.2 g), curcumin-treated by oral gavage (20.3 ± 0.2 g) and curcumin-treated by i.p. injection (21.9 ± 0.4 g) mice were statistically indistinguishable on the day of necropsy, and no biologically meaningful differences were evident in hematological examinations (Table 1).

4. Discussion

The transcription factor NF-κB is considered a master regulator of both innate and adaptive immune responses, and has been demonstrated to play a critical role in allergic airway disease. The first evidence to support the functional relevance of NF-κB in the development of allergic airway disease stemmed from observations in mice globally lacking members of the NF-κB family. These studies showed that the absence of p50 or c-Rel subunits of NF-κB protected mice against the development of allergic airway disease (Yang et al., 1998; Donovan et al., 1999). NF-κB activation occurs rapidly in the OVA-induced pulmonary inflammation model, and is predominantly observed in epithelial cells of the conducting airways (Poynter et al., 2002). The therapeutic potential of NF-κB

Table 1
Hematological analysis of the mice.

Group	WBC ($\times 10^6$/ml)	RBC ($\times 10^9$/ml)	Hemoglobin (mg/dl)
SAL	12.5 ± 0.2	9.3 ± 0.3	15.3 ± 0.5
OVA	9.3 ± 0.6	9.6 ± 0.2	15.1 ± 0.3
CMN-PO	10.7 ± 0.9	10.3 ± 0.2	14.9 ± 0.3
CMN-IP	11.3 ± 0.3*	9.1 ± 0.5	14.6 ± 0.5

* Significantly different from OVA group ($p < 0.05$).

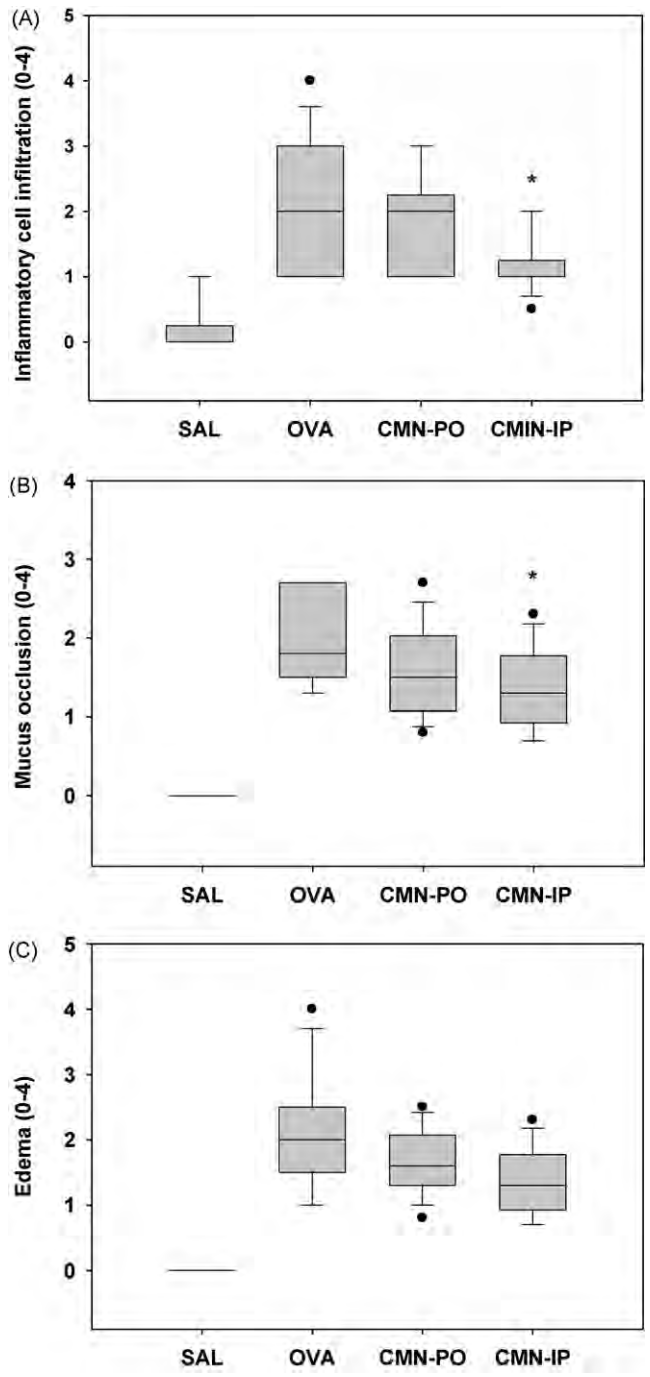

Fig. 5. Quantitative effects of curcumin on airway inflammation (grades). The grades of intensity of inflammatory cell infiltration (A), mucus occlusion of airways (B), and airway edema (C) are shown as the median values with percentile ranges. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. ($p < 0.05$ compared with the OVA control group.

inhibition in established asthma was demonstrated by delivering NF-κB decoy oligodeoxynucleotides to OVA-sensitized mice (Desmet et al., 2004). Interestingly, selective activation of NF-κB by the expression of an IκB kinase-β (IKKβ) transgene in airway epithelium is sufficient to induce AHR and smooth muscle thickening in the absence of antigen sensitization and challenge procedures (Pantano et al., 2008).

Curcumin has been shown to regulate numerous transcription factors, cytokines, and adhesion molecules, and modulate redox status. It is believed that the anti-inflammatory activity of turmeric is attributable to these various activities of curcumin. Curcumin has been shown to have anti-inflammatory effects in a number of disease models and was reported to inhibit LPS-induced production of TNF-α and IL-1 in the human monocytic macrophage cell line Mono Mac 6 (Chan, 1995). Curcumin has also been reported to inhibit LPS-induced activation of nuclear NF-κB. In the alveolar epithelial cell line A549, curcumin inhibits both H_2O_2- and TNF-α-mediated activation of NF-κB and AP-1, and IL-8 release (Biswas et al., 2005). Curcumin also inhibits the lymphocyte proliferation and production of IL-2, IL-5, and granulocyte macrophage-colony stimulating factor (GM-CSF) induced by house dust mites (*Dermatophagoides farinea*: *Df*), indicating that curcumin potentially modulates allergic disease by inhibiting the production of cytokines that affect eosinophil function and IgE synthesis (Kobayashi et al., 1997). Inhibition of NF-κB activation by curcumin is mediated by inhibition of IκBα kinase (Aggarwal et al., 2006) and AKT (Deeb et al., 2007), and results in the suppression of NF-κB-dependent gene products (Aggarwal and Harikumar, 2009).

In this study, we demonstrated the anti-NF-κB activity of curcumin in two cell lines associated with allergic airway disease. In A549 alveolar epithelial cells, curcumin inhibited the PMA-stimulated transcriptional activity of NF-κB. In murine macrophage-like Raw264.7 cells, treatment with curcumin stabilized IκBα, preventing its degradation and blocking nuclear translocation of p65 and p50. We confirmed the engagement of NF-κB activation in asthmatic lung tissue and demonstrated the effect of curcumin on NF-κB activation *in vivo*, showing that p65 nuclear translocation was significantly increased in asthmatic lung tissue 1 and 4 h (Fig. 6), but not 24 h (data not shown), after OVA challenge, indicating the rapid and early involvement of NF-κB activation in allergic inflammation after allergen exposure. Importantly, curcumin protected against OVA-induced AHR and significantly reduced OVA-induced inflammatory cell infiltration into the lung. Eosinophil numbers, in particular, were reduced, a decrease that was associated with a reduction in IgE level in BAL fluid. Elevated level of total IgE or allergen-specific IgE within the BAL fluid, sometimes independent of alterations in circulating IgE was reported in the asthmatic patients (Crimi et al., 1983; Peebles et al., 2001; Wilson et al., 2002) and also in animal models of asthma (Choi et al., 2005; Matheu et al., 2009). It was suggested that IgE measurements in BAL fluid may reflect ongoing local mucosal IgE synthesis which precede systemic synthesis (Wilson et al., 2002).

A limited number of reports have demonstrated the efficacy of curcumin in animal models of asthma. In sensitized guinea pigs, curcumin was shown to attenuate OVA-induced AHR, although the effect of curcumin on the airway inflammatory response was not reported in this study (Ram et al., 2003). In a mouse model of latex allergy, curcumin reduced the Th2 response concurrent with a reduction in pulmonary inflammation (Kurup et al., 2007). It has also been reported that curcumin attenuates both airway inflammation and AHR in a mouse model of OVA-induced asthma. The authors of this latter study suggested that suppression of inducible nitric oxide synthase (iNOS) and NO might be one mechanism underlying the anti-inflammatory action of curcumin (Moon et al., 2008). However, the authors concluded that the effect of curcumin on AHR was not mediated through NO because AHR has not been linked with NO in asthma.

In the current study, intraperitoneal administration of curcumin attenuated AHR and airway inflammation, and reduced IgE levels in BAL fluid of OVA-induced asthmatic mice. However, we found that daily oral administration of the same dose of curcumin (200 mg/kg/day) had no significant effect on these parameters although it showed tendency of minor amelioration. Curcumin was reported to be poorly absorbed from the gut (Ravindranath and

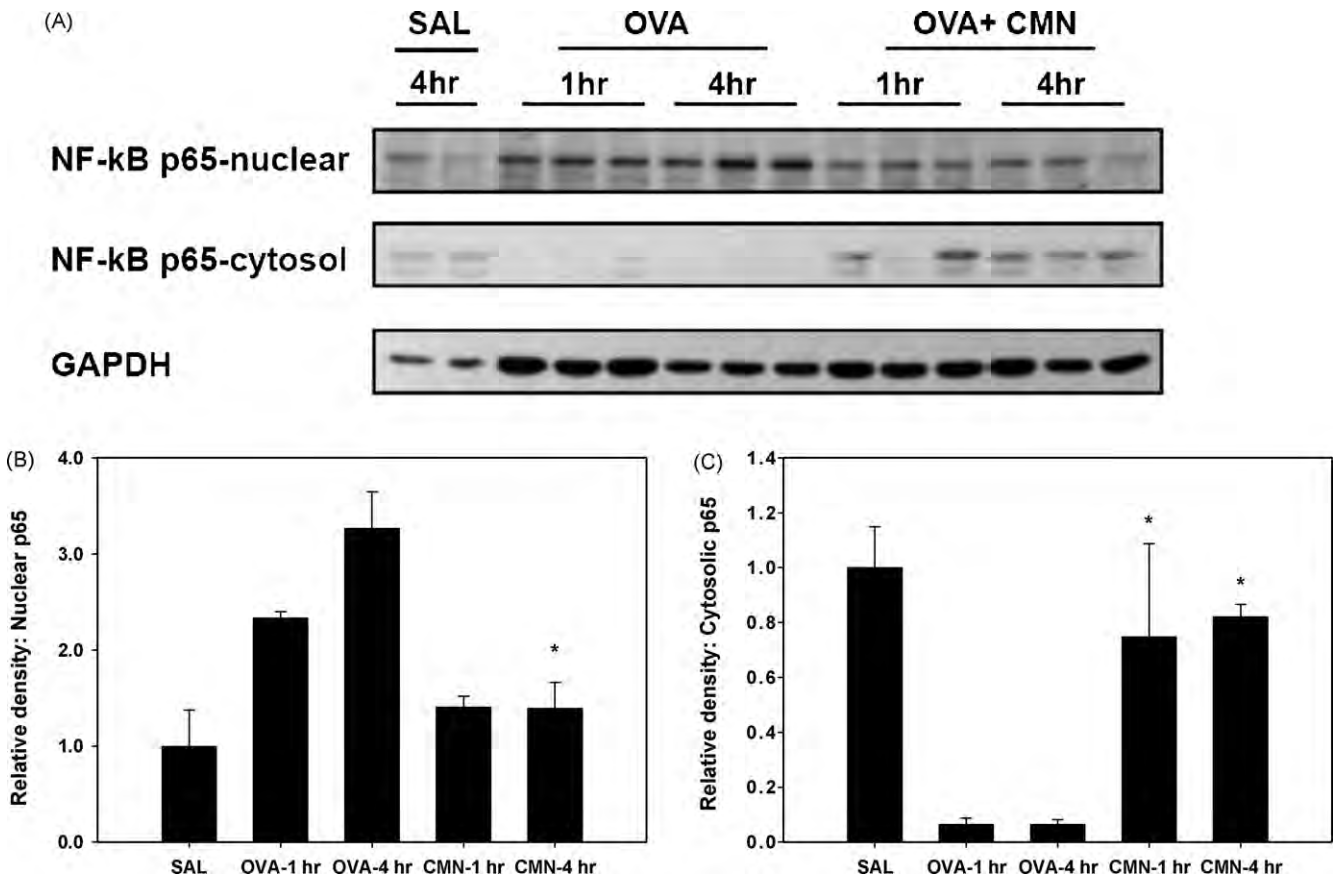

Fig. 6. Effect of curcumin on NF-κB activation in asthmatic lung tissue. Lung tissues collected 1 or 4 h after OVA challenge on day 15 from saline-treated (SAL, $n = 3$), OVA control (OVA, $n = 3$) and OVA + CMN-treated (OVA + CMN, $n = 3$) mice. p65 expression level in nuclear and cytosolic fractions was analyzed by Western blotting. (A) Western blot image, (B) quantified expression level of p65 in the nucleus normalized against GADPH, (C) quantified expression level of p65 in the cytoplasm normalized against GADPH. The values shown are the means ± SEMs. ($p < 0.05$ compared with the OVA control group.

Chandrasekhara, 1980, 1982), and maximum concentrations in the plasma were considerably higher after intraperitoneal injection than after oral administration in BALB/c mice (Pan et al., 1999), indicating that the poor oral efficacy we observed may be due to reduced absorption of orally administered curcumin. The marked difference of efficacy by different route of administration in this study suggests that concomitant administration of curcumin with glucuronidation inhibitor such as piperine (Shoba et al., 1998) may be useful to enhance efficacy of curcumin through improvement of oral bioavailability.

The significant inhibitory effect of curcumin on p65 translocation *in vivo*, demonstrated here, suggests that the anti-inflammatory effect of curcumin is attributable, at least in part, to its anti-NF-κB activity. Furthermore, considering that selective activation of NF-κB within the airway epithelium is sufficient to cause AHR in mice (Pantano et al., 2008), the anti-AHR effect of curcumin demonstrated in this study could be partially attributable to the anti-NF-κB activity of curcumin. This is the first report demonstrating the efficacy of curcumin in a murine model of asthma through a mechanism that possibly involves inhibition of NF-κB activation. The data presented here indicate that curcumin may be beneficial for the prevention and alleviation of asthma and related airway diseases.

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