



Mangosteen xanthones mitigate ovalbumin-induced airway inflammation in a mouse model of asthma

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ABSTRACT

α - and γ -Mangostin, which are the major xanthones purified from a Mangosteen, *Garcinia mangostana* Linn., exhibit a wide range of anticancer, antioxidant, and anti-inflammatory activities. Here, we assessed their therapeutic effects in a mouse model of ovalbumin (OVA)-induced allergic asthma. Animals were treated with α - and γ -mangostins orally for 3 days at doses of 10 and 30 mg/kg daily, 1 h before the OVA challenge. Administration of α - and γ -mangostins significantly reduced the major pathophysiological features of allergic asthma, including inflammatory cell recruitment into the airway, airway hyper-responsiveness (AHR), and increased levels of Th2 cytokines. In addition, α - and γ -mangostins attenuated the increases in phosphoinositide 3-kinase (PI3K) activity, phosphorylation of Akt, and NF- κ B in nuclear protein extracts after OVA challenge. In conclusion, α - and γ -mangostin may have therapeutic potential for the treatment of allergic asthma.

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1. Introduction

Allergic asthma is a chronic inflammatory disease characterized by airway infiltration of inflammatory cells inducing airway hyper-responsiveness (AHR) and structural remodeling of the airways. Allergic airway inflammation and AHR development involve multiple inflammatory cell types and a wide array of mediators. Recent studies suggested that numerous components of the PI3K pathway play a crucial role in the expression and activation of inflammatory mediators, inflammatory cell recruitment, immune cell function, airway remodeling, and corticosteroid insensitivity in inflammatory respiratory disease (Medina-Tato et al., 2007). PI3K activity is stimulated after antigen challenge in a murine model of allergic asthma, and administration of wortmannin (Ezeamuzie et al., 2001) or LY294002 (Kwak et al., 2003), two broad spectrum inhibitors of PI3K, significantly inhibits most of the pathophysiological features of the asthma, including increased eotaxin, IL-5, and IL-13 levels in BAL fluids, eosinophil infiltration of airway, airway

mucus production, and AHR (Kwak et al., 2003). Thus, blockade of PI3K signaling enzymes might be effective in reducing allergic airway inflammation (Ezeamuzie et al., 2001).

α - and γ -Mangostins are the major xanthones purified from a mangosteen, *Garcinia mangostana* Linn, which has been used as a traditional medicine for the treatment of trauma, diarrhea, wound infection, pain, fever, and convulsion (Cui et al., 2010). α -Mangostin has been shown to induce apoptosis and inhibit cell proliferation through down-regulation of mitogen-activated protein (MAP) kinases and Akt signaling in human chondrosarcoma (Krajarng et al., 2011), colon cancer (Nakagawa et al., 2007), and breast cancer cells (Moongkarndi et al., 2004). Also, α -mangostin is a competitive antagonist of the histamine H₁ receptor (Chairungsrilerd et al., 1996) and possesses many biological effects, such as anticancer (Akao et al., 2008), antioxidant (Chen et al., 2008), and anti-inflammatory activities (Tewtrakul et al., 2009). γ -Mangostin has been reported to exert inhibitory effects on 5-HT_{2A} and 5-HT_{2C} receptors in the peripheral nervous system and central nervous system (Chairungsrilerd et al., 1998), and an anti-inflammatory effect in C6 rat glioma cells (Nakatani et al., 2004). Recently, it was reported that α - and γ -mangostins inhibited the expression of cytokines related to allergic and inflammatory disease in mast cells (Chae et al., 2012), implying these compounds may have potential to alleviate allergic responses in asthmatic models. Since there is no report regarding the anti-asthmatic effects of α - and γ -mangostin on allergic asthma, we investigated the effect of α - and γ -mangostin, on

Abbreviations: AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; ELISA, enzyme-linked immunosorbent assay; NF- κ B, nuclear factor-kappa B; OVA, ovalbumin; PAS, periodic acid-Schiff; SAL, saline.

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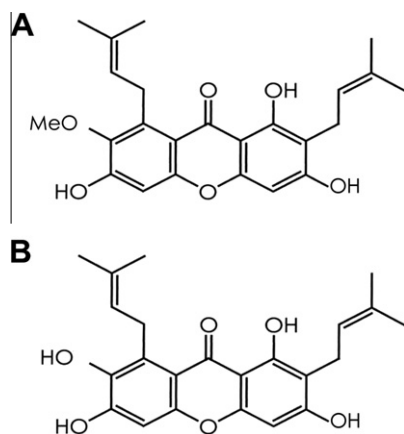


Fig. 1. (A), The chemical structure of α -mangostin; molecular formula, $C_{24}H_{26}O_6$; molecular weight, 410.46. (B), The chemical structure of γ -mangostin; molecular formula, $C_{23}H_{24}O_6$; molecular weight, 396.43.

airway inflammation, AHR, Th2 cytokines, and PTEN/PI3K pathway. Our findings suggest that α - and γ -mangostin possess therapeutic potential for the treatment of allergic asthma.

2. Materials and methods

2.1. Experimental regimen

The percarp of *G. mangostana* L. were collected in Indonesia. The separation and identification of α - and γ -mangostin were conducted as described elsewhere (Quan et al., 2010). The purity of α - and γ -mangostin was determined to be >95% based on HPLC-UV analysis. The chemical structures of α - and γ -mangostin are depicted in Fig. 1.

2.2. Animals

Female BALB/c mice, 6 weeks of age (Koatech Co., Korea), were used under protocols approved (KRIBB-AEC-11132) by the Institutional Animal Care and Use Committee of the Korea Research Institute of Bioscience and Biotechnology.

2.3. Experimental protocol

Specific-pathogen-free female BALB/c mice were sensitized on days 1 and 14 by intraperitoneal injection of 20 μ g OVA (Sigma A5503; Sigma-Aldrich, St. Louis, MO, USA), emulsified in 2 mg aluminum hydroxide (Sigma-Aldrich) in a total volume of 200 μ l. On days 21, 22, and 23 after initial sensitization, the mice were challenged for 30 min with an aerosol of 1% (wt/vol) OVA in saline using an ultrasonic nebulizer (NE-U12; Omron Corp., Tokyo, Japan). Control mice were sensitized and challenged using the same protocol but using only saline. All samples (α -mangostin, γ -mangostin, dexamethasone, montelukast) were dissolved in PBS and each sample (0.2 ml/mouse) was treated. One hour before each challenge, α -mangostin (10 or 30 mg/kg body weight/day) or γ -mangostin (10 or 30 mg/kg body weight/day) was administered by oral gavage for three days. For a drug control, mice treated with dexamethasone (3 mg/kg body weight/day; Sigma-Aldrich) or montelukast (30 mg/kg body weight/day) was administered in the same manner. Bronchoalveolar lavage (BAL) was performed at 48 h after the last challenge.

2.4. Inflammatory cell counts in bronchoalveolar lavage fluid

BAL was performed as described previously (Lee et al., 2006). BAL fluid was obtained from mice anesthetized with 50 mg/kg of pentobarbital (Hanlim Pharm., Korea) by lavaging lungs with saline delivered via a tracheal cannula. BAL fluid was deposited onto cytospin slides and stained with Diff-Quik (Dade Behring Inc., Deerfield, IL, USA). Differential cell counts were performed by two independent investigators.

2.5. Measurement of airway responsiveness

Airway responsiveness was assessed 24 h after the last challenge by indirectly using single-chamber, whole body plethysmography (Allmedicus, Seoul, Korea). Each conscious mouse was challenged with methacholine aerosols in increasing concentrations (12.5–50 mg/ml in saline) for 3 min. After each methacholine challenge, penh values were calculated for 3 min.

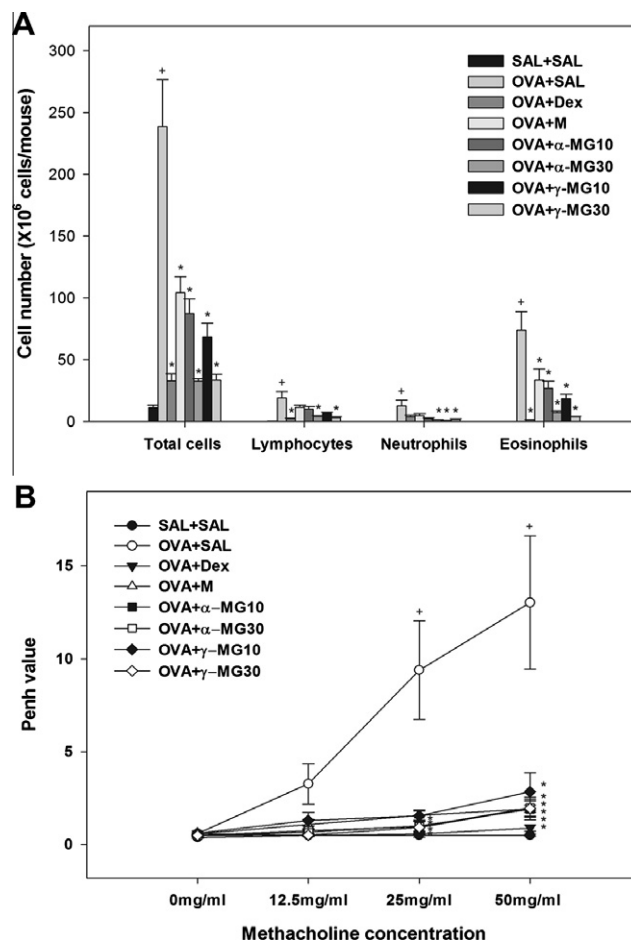


Fig. 2. (A), Effect of α - and γ -mangostin on total cells and differential cellular components in BAL fluids. BAL was performed at 48 h after the last challenge in the following groups: control mice challenged and administered saline (SAL + SAL), OVA challenged mice administered saline (OVA + SAL), OVA challenged mice administered dexamethasone (OVA + Dex), OVA challenged mice administered montelukast (OVA + M), OVA challenged mice administered 10 mg/kg α -mangostin (OVA + α -MG10), OVA challenged mice administered 30 mg/kg of α -mangostin (OVA + α -MG30), OVA challenged mice administered 10 mg/kg of γ -mangostin (OVA + γ -MG10), or OVA challenged mice administered 30 mg/kg of γ -mangostin (OVA + γ -MG30). (B), Effect of α - and γ -mangostin on airway responsiveness. Data represent the mean $\pm$ SEM from five mice per group. * p < 0.05 vs. SAL + SAL; ** p < 0.05 vs. OVA + SAL.

2.6. Histology

At 48 h after the last challenge, lungs were removed from the mice after sacrifice. Lungs were fixed with 4% paraformaldehyde prior to embedding in paraffin. Sections (4 μ m thick) were stained with hematoxylin and eosin to assess inflammatory cell infiltration. Inflammation was evaluated using a semi-quantitative scoring system with a grading scale ranging from 0 to 3. A value of 0 was assigned when no inflammation was detectable; (1) was assigned when occasional cuffing with inflammatory cells was observed; (2) was assigned when most bronchi or vessels were surrounded by a thin layer (one to five cells) of inflammatory cells; and a value of (3) was assigned when most bronchi or vessels were surrounded by a thick layer (>5 cells) of inflammatory cells.

2.7. Quantitation of airway mucus expression

Goblet cell hyperplasia and mucus expression were assessed by periodic acid-Schiff (PAS) staining. Mucus expression levels in the airway were quantified by counting PAS-positive and PAS-negative epithelial cells in each bronchiole, as described previously. Results are expressed as the percentage of PAS-positive cells per bronchiole, which was calculated as the number of PAS-positive epithelial cells per bronchiole divided by the total number of epithelial cells in each bronchiole.

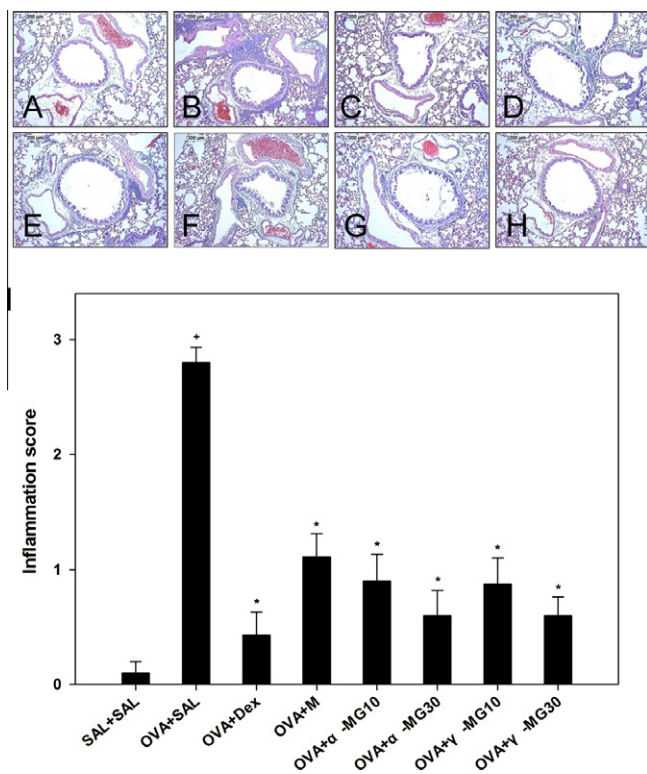


Fig. 3. Effect of α - and γ -mangostin in lung tissues of OVA-challenged mice. (A–H) (original magnification X100), (H and E) stained lung sections of control mice. (A), OVA challenged mice administered saline. (B), OVA challenged mice administered dexamethasone. (C), OVA challenged mice administered montelukast. (D), OVA challenged mice administered 10 mg/kg of α -mangostin. (E), OVA challenged mice administered 30 mg/kg of α -mangostin. (F), OVA challenged mice administered 10 mg/kg of γ -mangostin. (G), or OVA challenged mice administered 30 mg/kg of γ -mangostin (H). (I), lung inflammation was measured, and total lung inflammation was defined as the average of the inflammation scores. Data represent the mean $\pm$ SEM from five mice per group. * $p < 0.05$ vs. SAL + SAL; * $p < 0.05$ vs. OVA + SAL.

2.8. Quantitation of peribronchial fibrosis

Masson's trichrome staining was used to assess peribronchial fibrosis. Image Pro. Plus (Media Cybernetics Company, Silver Spring, MD, USA) was used to outline and quantify the airway lumen perimeter and the area of collagen staining around bronchi and vessels. The areas of trichrome staining for collagen are expressed relative to the length of basement membrane of bronchioles 150–200 μ m of internal diameter (μ m²/ μ m), as described previously (Lee et al., 2008). At least 10 bronchioles were counted in each slide.

2.9. Western blot analysis

Lung tissues were homogenized in the presence of protease inhibitors to obtain extracts of lung proteins. Protein concentrations were determined using the Bradford reagent (Bio-Rad Inc., Hercules, CA). Samples (20 μ g protein per lane) were loaded on a 10% SDS-PAGE gel. After electrophoresis, separated proteins were transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech., Piscataway, NJ) by the wet transfer method (250 mA, 90 min). Nonspecific sites were blocked with 5% nonfat milk in Tris-buffered saline Tween 20 (TBST; 25 mM Tris pH 7.5, 150 mM NaCl, 0.1% Tween 20) for 2 h, and the blots were then incubated with an anti-Akt antibody (Cell Signaling Technology Inc., Beverly, MA, USA), anti-phosphorylated Akt (p-Akt) antibody (Cell Signaling Technology Inc.), anti-PTEN antibody (Cell Signaling Technology Inc.), or anti-TGF- β 1 antibody (Cell Signaling Technology Inc.) overnight at 4 °C. Anti-rabbit horseradish peroxidase-conjugated IgG was used to detect binding of the antibodies. The binding of the specific antibody was visualized by exposing membrane to photographic film after treating them with enhanced chemiluminescence system reagents (Amersham Pharmacia Biotech.).

2.10. Cytosolic or nuclear protein extraction for analysis of NF- κ B p65

Both nuclear and cytoplasmic extracts from mouse lung were prepared with an NE-PER Nuclear and Cytoplasmic Extraction Reagent kit (Pierce, Rockford, IL, USA) according to the manufacturer's instructions for analysis of NF- κ B p65. The levels

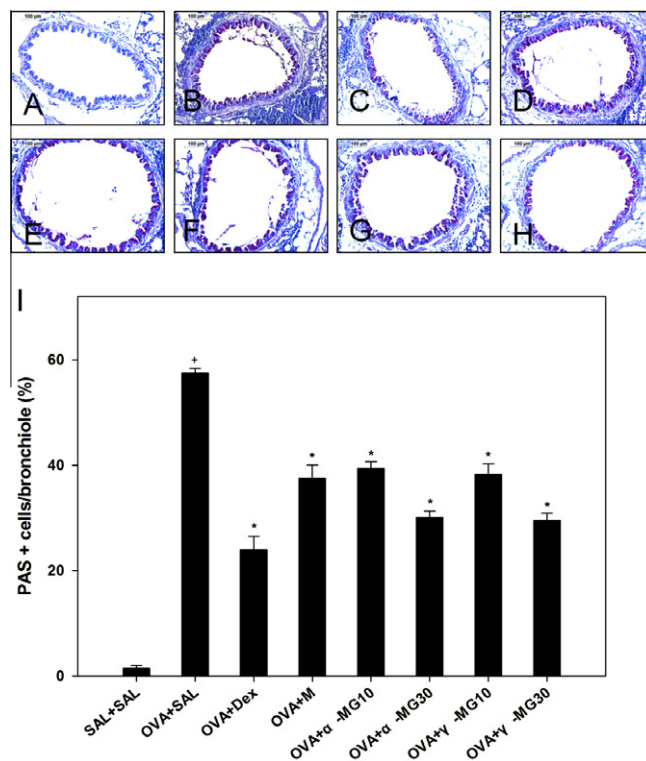


Fig. 4. Effect of α - and γ -mangostin on airway mucus expression. (A–H) (original magnification X200), PAS-stained lungs sections of control mice. (A), OVA challenged mice administered saline. (B), OVA challenged mice administered dexamethasone. (C), OVA challenged mice administered montelukast. (D), OVA challenged mice administered 10 mg/kg of α -mangostin. (E), OVA challenged mice administered 30 mg/kg of α -mangostin. (F), OVA challenged mice administered 10 mg/kg of γ -mangostin. (G), or OVA challenged mice administered 30 mg/kg of γ -mangostin (H). (I), Quantitation of airway mucus expression. Data represent the mean $\pm$ SEM from five mice per group. * $p < 0.05$ vs. SAL + SAL; * $p < 0.05$ vs. OVA + SAL.

of NF- κ B p65 were detected using an anti-p65 antibody (Cell Signaling Technology, Beverly, MA, USA). Densitometric band values were determined using Image J. Software from the National Institute of Health.

2.11. Measurement of PI3K enzyme activity in lung tissue

Lung tissues were homogenized in the presence of protease inhibitors to obtain extracts of lung proteins. Protein concentrations were determined using the Bradford reagent (Bio-Rad). The amount of phosphatidylinositol-3,4,5-triphosphate (PIP3) produced was quantified by PIP3 competition enzyme immunoassays according to the manufacturer's protocol (Echelon, Inc., Salt Lake City, UT). The enzyme activity was expressed as pmol of PIP3 produced by 1 ml of lung tissue extracts containing equal amounts of total protein.

2.12. Measurement of total IgE and OVA-specific IgE

Serum and BAL fluid levels of total and OVA-specific IgE were measured by enzyme-linked immunosorbent assay (ELISA). Briefly, microtiter plates were coated with 100 μ l/well IgE (2 μ g/ml; Serotec, Oxford, UK) and OVA (10 μ g/ml; Sigma-Aldrich) in PBS/Tween-20. Antibodies in serum and BAL fluid were detected using isotype-specific secondary antibodies (anti-mouse IgE; Serotec, Oxford, UK). After washing four times, 200 μ l of *o*-phenylenediamine dihydrochloride (Sigma-Aldrich) was added to each well. The plate was incubated for 10 min in the dark, and absorbance was measured at 450 nm. Total and OVA-specific IgE concentrations were calculated from a standard curve generated from a 250 ng/ml solution of recombinant IgE (Serotec, Oxford, UK).

2.13. Measurement of Th2 cytokines and TGF- β 1

Levels of IL-4, IL-5, IL-13, and TGF- β were quantified in the supernatant of BAL fluids by enzyme immunoassays (BioSource International, Camarillo, CA, USA) according to the manufacturer's instructions.

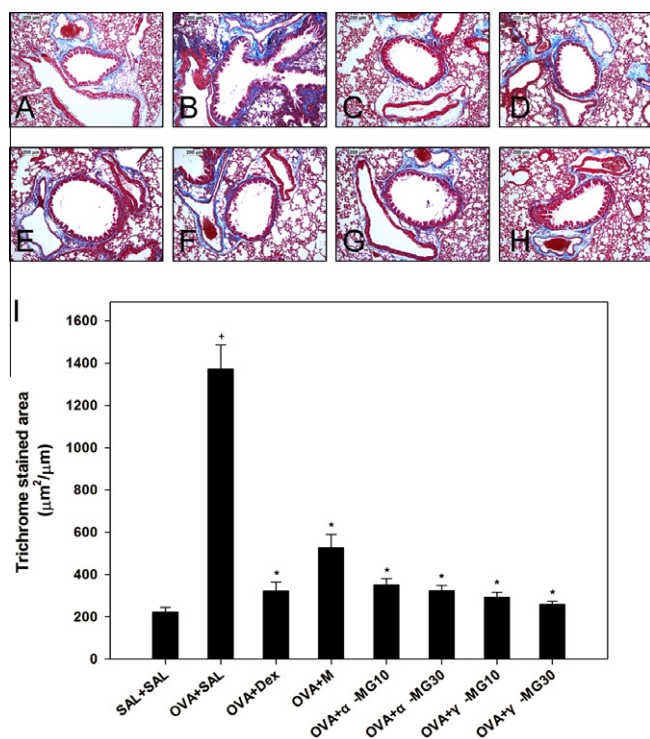


Fig. 5. Effect of α - and γ -mangostin on peribronchial fibrosis in lung tissues. (A–H) (original magnification X100), trichrome stained lung sections of control mice. (A), OVA challenged mice administered saline. (B), OVA challenged mice administered dexamethasone. (C), OVA challenged mice administered montelukast. (D), OVA challenged mice administered 10 mg/kg of α -mangostin. (E), OVA challenged mice administered 30 mg/kg of α -mangostin. (F), OVA challenged mice administered 10 mg/kg of γ -mangostin. (G), or OVA challenged mice administered 30 mg/kg of γ -mangostin (H). (I), Quantitation of peribronchial fibrosis. Data represent the mean $\pm$ SEM from five mice per group. * $p < 0.05$ vs. SAL + SAL; * $p < 0.05$ vs. OVA + SAL.

2.14. Densitometric analyses and statistics

The data are expressed as mean $\pm$ SE. SPSS 12.0 soft-ware was used for statistical analyses. Differences between groups were determined by one-way ANOVA. A p -value less than 0.05 was considered statistically significant.

3. Results

3.1. Effect of α - and γ -mangostin on cellular changes in BAL fluids

The numbers of total cells, lymphocytes, neutrophils, and eosinophils were significantly increased in BAL fluid at 48 h after the last OVA challenge compared with levels in control mice (Fig. 2A). The increased numbers of total cells, lymphocytes, neutrophils, and eosinophils in BAL fluids after the last OVA challenge were significantly reduced by the administration of dexamethasone, montelukast, α -, and γ -mangostin.

3.2. Effect of α - and γ -mangostin on airway hyperresponsiveness

Airway responsiveness was assessed as penh values in response to increasing doses of methacholine. The penh values produced by methacholine administration (at doses from 12.5 to 50 mg/ml) increased significantly in OVA-sensitized and -challenged mice compared with control mice. OVA-sensitized and -challenged mice treated with α - and γ -mangostin showed significantly decreased penh values in response to increased doses of methacholine compared with control mice (Fig. 2B). These results indicated that

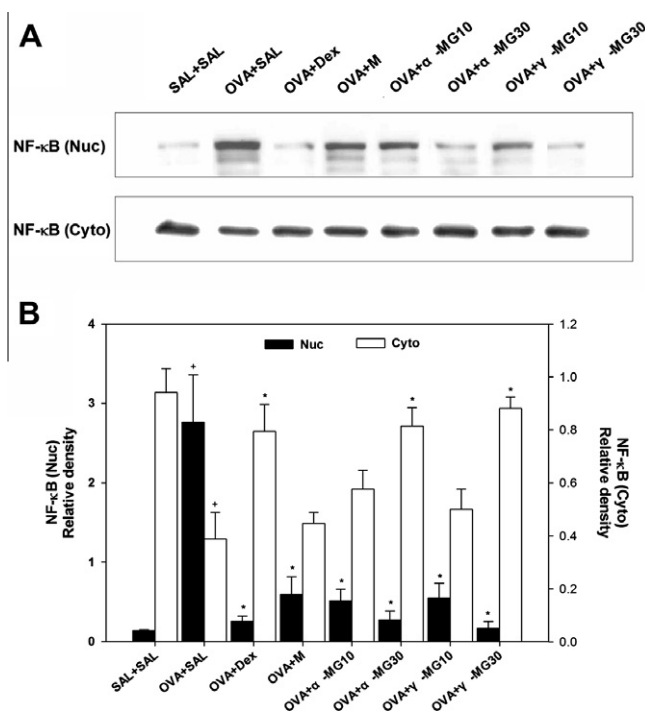


Fig. 6. Effect of α - and γ -mangostin on NF- κ B expression in nuclear and cytosolic protein extracts from lung tissues. (A), Western blotting of NF- κ B p65 in nuclear (Nuc) and cytosolic (Cyto) protein extracts from lung tissues. (B), Densitometric analyses are presented as the relative ratio of NF- κ B (Nuc) to PARP and NF- κ B (Cyto) to actin. Data represent the mean $\pm$ SEM from five mice per group. * $p < 0.05$ vs. SAL + SAL; * $p < 0.05$ vs. OVA + SAL.

dexamethasone, montelukast, α -, and γ -mangostin treatment reduced OVA-induced airway hyperresponsiveness.

3.3. Effect of α - and γ -mangostin on pathological changes

Histological analysis revealed typical pathological features of asthma in the OVA-sensitized and -challenged mice. Numerous inflammatory cells infiltrated around the bronchiole, the airway epithelium was thickened, and mucus and debris had accumulated in the lumen of bronchioles (Fig. 3B) as compared with control mice (Fig. 3A). Mice treated with α -mangostin (Fig. 3E and F) or γ -mangostin (Fig. 3G and H) showed marked reduction in the thickening of the airway epithelium, in the infiltration of inflammatory cells in the peribronchiolar region in the number of inflammatory cells, and in the amount of debris in the airway lumen. Dexamethasone (Fig. 3C) and montelukast (Fig. 3D) also significantly decreased inflammatory cells infiltration in the lung. The scores of lung inflammation were significantly increased at 48 h after the last OVA challenge compared with scores in control mice. The increased lung inflammation after OVA challenge was significantly decreased by the administration of α - and γ -mangostin (Fig. 3I).

3.4. Effect of α - and γ -mangostin on airway mucus expression

The percentage of airway epithelium that stained positively with PAS in mice repetitively challenged with OVA (Fig. 4B) was significantly greater than in control mice (Fig. 4A). The administration of α - (Fig. 4E and F) and γ -mangostin (Fig. 4G and H) to OVA-sensitized and -challenged mice significantly reduced the percentage of airway epithelium staining positively with PAS

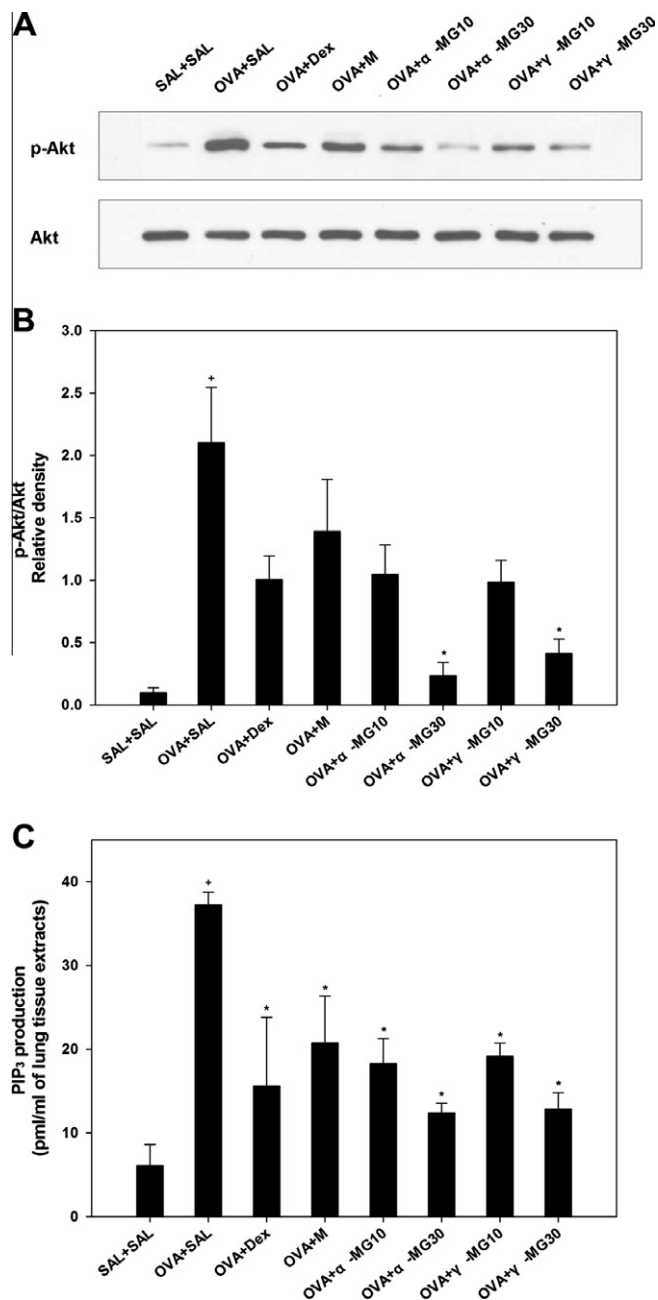


Fig. 7. Effect of α - and γ -mangostin on p-Akt and Akt protein levels, and PI3K enzyme activity. (A), Western blotting of p-Akt and Akt in lung tissues. (B), Densitometric analyses are presented as the relative ratio of p-Akt to Akt. (C), Enzyme immunoassay of PIP3 production by PI3Ks in lung tissue extracts. Data represent the mean $\pm$ SEM from five mice per group. * $p < 0.05$ vs. SAL + SAL; * $p < 0.05$ vs. OVA + SAL.

compared with untreated mice, and it was similar to the reduction in dexamethasone (Fig. 4C) and montelukast (Fig. 4D) treated mice.

3.5. Effect of α - and γ -mangostin on peribronchial collagen deposition

Mice exposed to repetitive OVA challenge (Fig. 5B) had a significant increase in the levels of peribronchial fibrosis compared with control mice (Fig. 5A) as assessed by trichrome staining. The administration of α - (Fig. 5E and F) and γ -mangostin (Fig. 5G and H) to OVA-sensitized and -challenged mice significantly reduced the levels of peribronchial fibrosis compared with untreated mice,

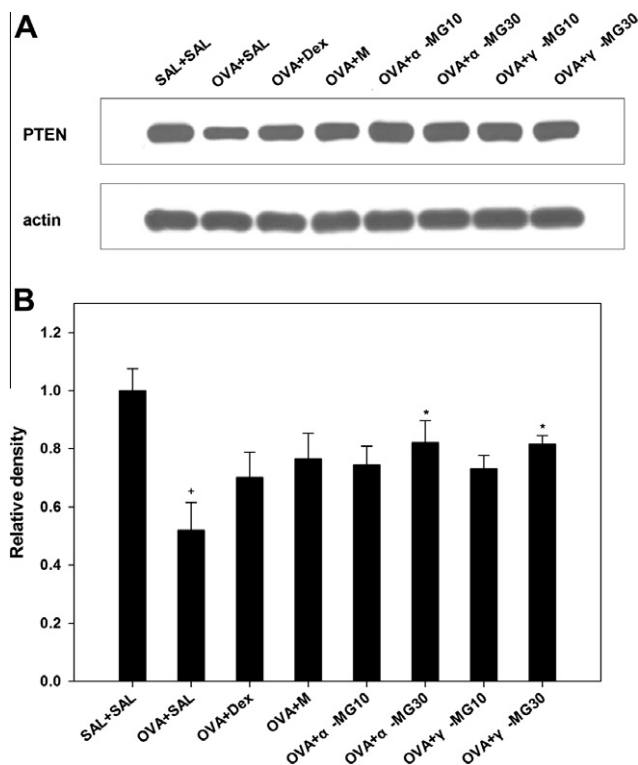


Fig. 8. Effect of α - and γ -mangostin on PTEN protein expression in lung tissues of OVA-challenged mice. (A), Western blotting of PTEN. (B), Densitometric analyses are presented as the relative ratio of PTEN to actin. Data represent the mean $\pm$ SEM from five mice per group. * $p < 0.05$ vs. SAL + SAL; * $p < 0.05$ vs. OVA + SAL.

and it was similar to the reduction in dexamethasone (Fig. 5C) and montelukast (Fig. 5D) treated mice.

3.6. Effect of α - and γ -mangostin on NF- κ B p65 protein levels in lung tissue

Western blot analysis showed that levels of NF- κ B p65 in nuclear protein extracts from lung tissues were increased at 48 h after the last OVA challenge compared with levels in control mice (Fig. 6A and B). The increased NF- κ B p65 levels in nuclear protein extracts were decreased by the administration of α - and γ -mangostin. In contrast, the levels of NF- κ B p65 protein levels in cytosol fractions from lung tissues were decreased at 48 h after the last OVA challenge as compared with the levels in control mice. The decrease in NF- κ B p65 protein levels in the cytosol fraction from lung tissues were increased by the administration of α - and γ -mangostin.

3.7. Effect of α - and γ -mangostin on Akt phosphorylation and PI3K enzyme activity in lung tissue

The levels of p-Akt protein in lung tissues were increased significantly at 48 h after the last OVA challenge compared with levels in control mice (Fig. 7A and B). In contrast, no significant changes in total Akt protein levels were observed in any of the groups. The increased p-Akt, but not Akt, protein levels in lung tissues at 48 h after OVA challenge were significantly reduced by the administration of α - and γ -mangostin. Consistent with these results, PIP3 levels in lung tissues were increased at 48 h after the last OVA challenge compared with control mice (Fig. 7C). The increase in PIP3 levels in lung tissues was significantly attenuated by the administration of α - and γ -mangostin.

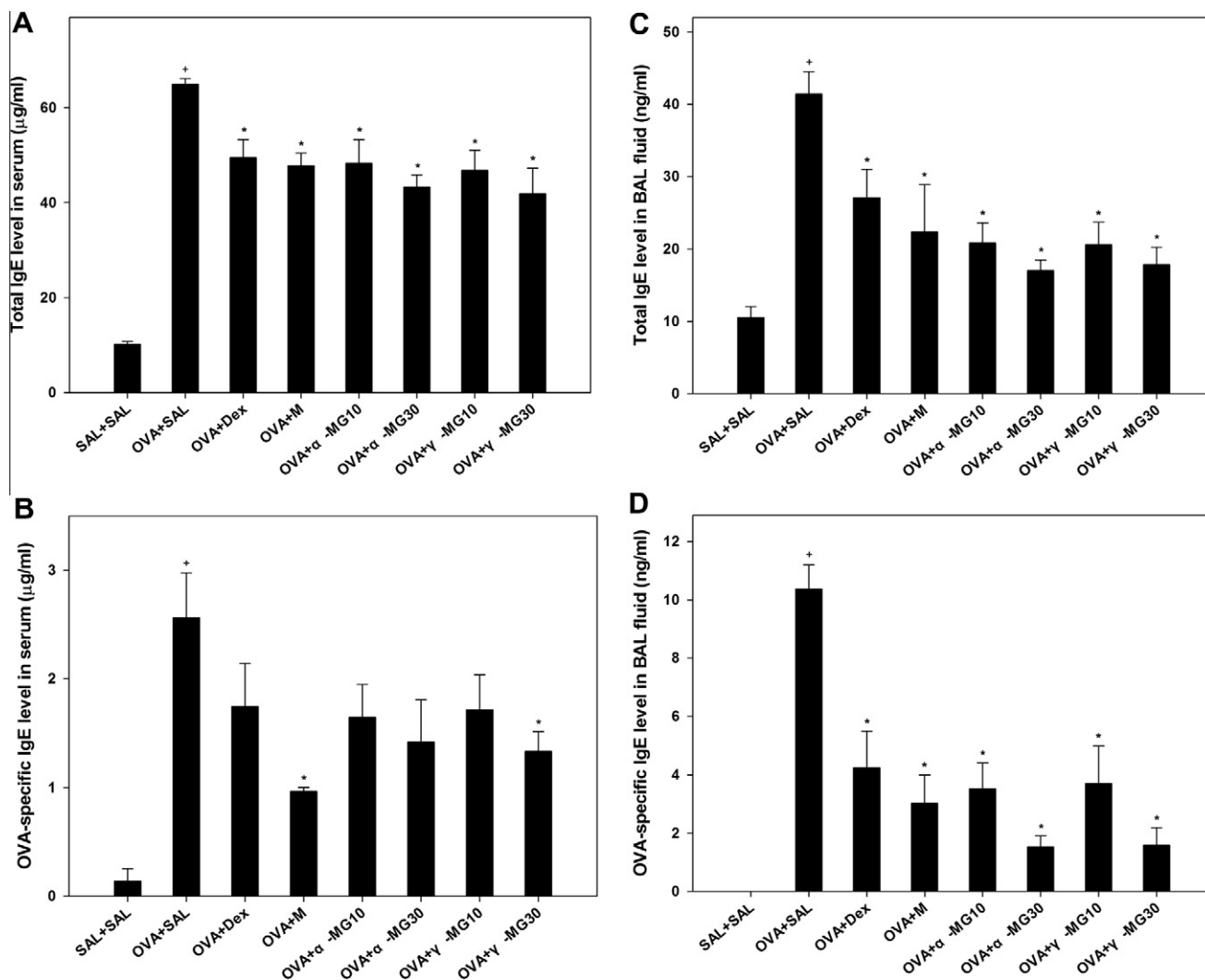


Fig. 9. Effect of α - and γ -mangostin on IgE and OVA-specific IgE levels in serum and BAL fluids. (A–D), Enzyme immunoassay of IgE in serum. (A), OVA-specific IgE in serum. (B), IgE in BAL fluids. (C), and OVA-specific IgE in BAL fluids (D). Data represent the mean $\pm$ SEM from five mice per group. * $p < 0.05$ vs. SAL + SAL; * $p < 0.05$ vs. OVA + SAL.

3.8. Effect of α - and γ -mangostin on PTEN protein levels in lung tissues

Western blot analysis revealed that PTEN protein levels were decreased significantly after OVA challenge compared with control mice (Fig. 8A and B). The decrease in PTEN levels after OVA challenge was increased substantially by the administration of 30 mg/kg of α - and γ -mangostin.

3.9. Effect of α - and γ -mangostin on levels of total IgE and OVA-specific IgE in serum and BAL fluids

Enzyme immunoassays showed that levels of total IgE and OVA-specific IgE in serum (Fig. 9A and B) and BAL fluids (Fig. 9C and D) were significantly increased at 48 h after the last OVA challenge compared with control mice. The increase in levels of total IgE and OVA-specific IgE was significantly attenuated by the administration of α - and γ -mangostin. Dexamethasone and montelukast also significantly reduced the levels of total IgE and OVA-specific IgE in serum.

3.10. Effect of α - and γ -mangostin on levels of IL-4, IL-5, and IL-13 in BAL fluids

Enzyme immunoassays showed that levels of IL-4 (Fig. 10A), IL-5 (Fig. 10B), and IL-13 (Fig. 10C) in BAL fluids were significantly

increased at 48 h after the last OVA challenge compared with control mice. The increase in levels of these cytokines was significantly attenuated by the administration of α - and γ -mangostin and it was similar to the reduction in dexamethasone and montelukast treated mice.

3.11. Effect of α - and γ -mangostin on TGF- β 1 expression in lung tissues and BAL fluids

Western blot analysis showed that TGF- β 1 protein levels in lung tissues were significantly increased 48 h after the last OVA challenge compared with levels in control mice (Fig. 11A and B). The increase in TGF- β 1 levels was significantly attenuated by the administration of α - and γ -mangostin. Consistent with results obtained from the western blot analysis, enzyme immunoassays revealed that levels of TGF- β 1 in BAL fluids were significantly increased at 48 h after the last OVA challenge compared with levels in the control mice (Fig. 11C). The increase in TGF- β 1 levels was significantly attenuated by the administration of α - and γ -mangostin.

4. Discussion

In this study, we show that administration of α - and γ -mangostin attenuated OVA-induced airway inflammation, Th2 cytokines

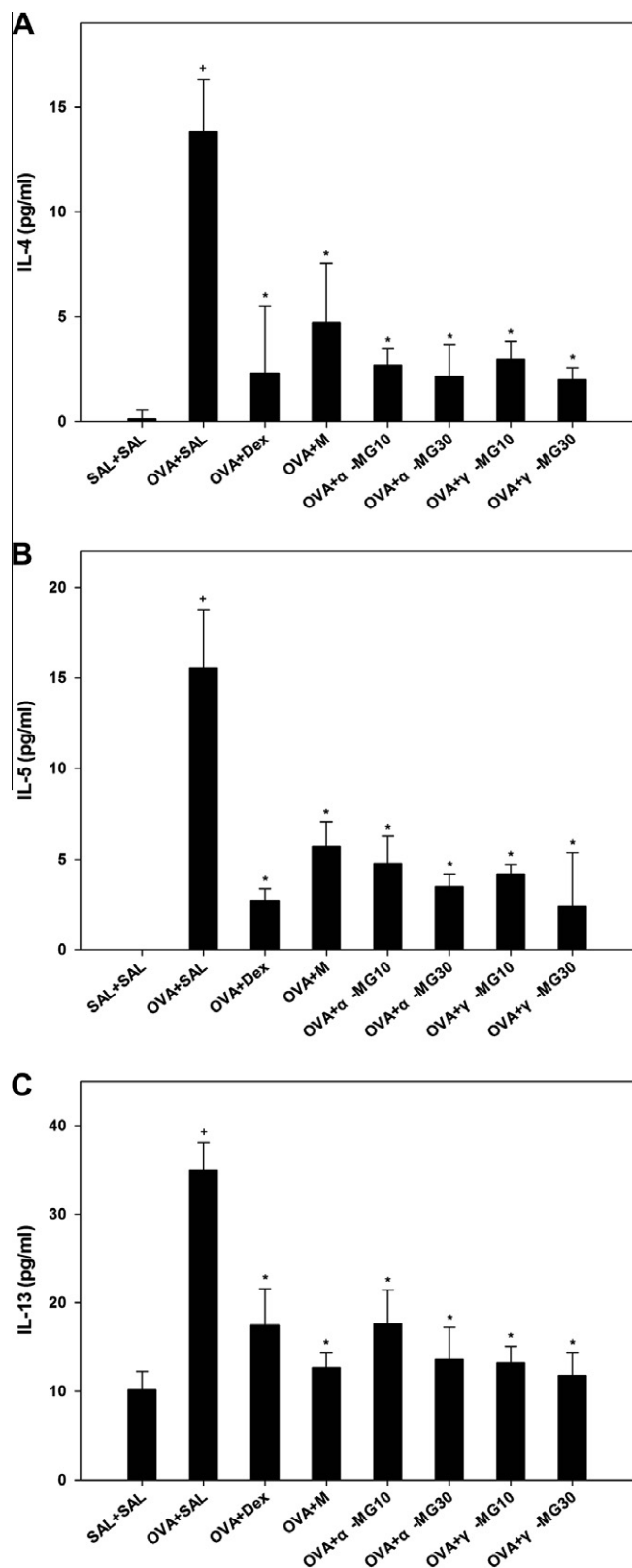


Fig. 10. Effect of α - and γ -mangostin on IL-4, IL-5, IL-13 levels in BAL fluids. (A–C), Enzyme immunoassay of IL-4 (A), IL-5 (B), and IL-13 (C) in BAL fluids. Data represent the mean $\pm$ SEM from five mice per group. * $p < 0.05$ vs. SAL + SAL; * $p < 0.05$ vs. OVA + SAL.

levels, IgE and OVA-specific IgE levels, the number of mucus-producing cells in the airway, peribronchial fibrosis, and AHR. Further-

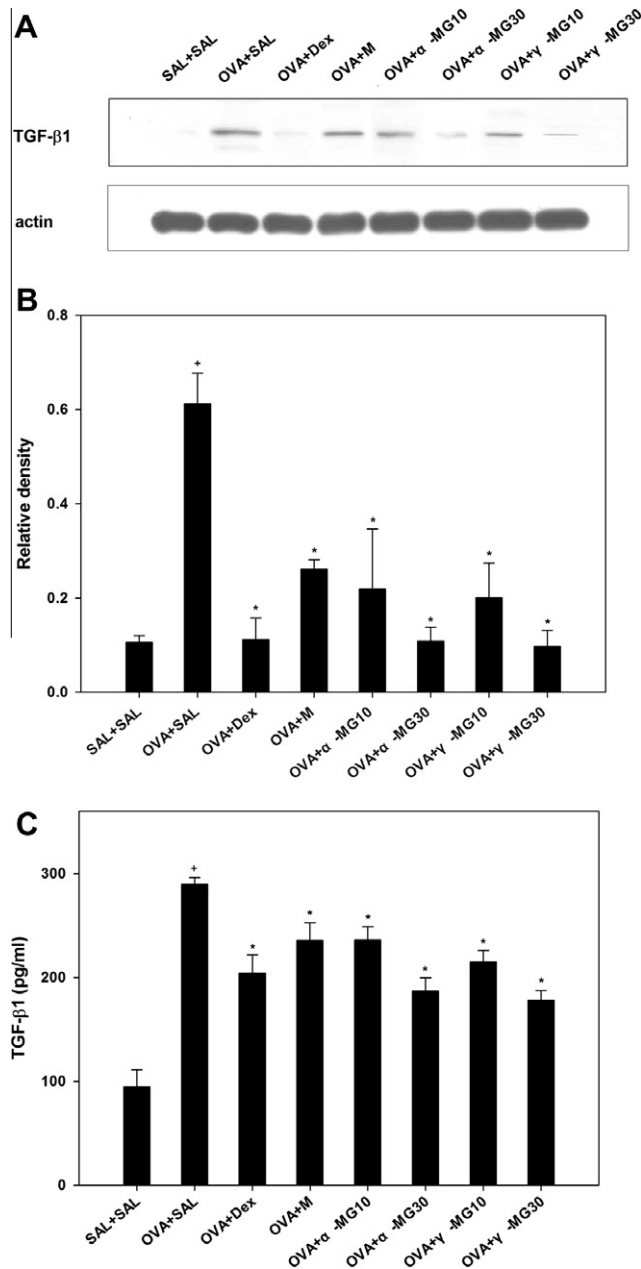


Fig. 11. Effect of α - and γ -mangostin on TGF- β 1 expression in lung tissues and in BAL fluids. (A), Western blotting of TGF- β 1. (B), Densitometric analyses are presented as the relative ratio of TGF- β 1 to actin. (C), Enzyme immunoassay of TGF- β 1 in BAL fluids. Data represent the mean $\pm$ SEM from five mice per group. * $p < 0.05$ vs. SAL + SAL; * $p < 0.05$ vs. OVA + SAL.

more, we show that these effects were associated with changes in the expression levels of signaling molecules from the Akt/NF- κ B pathway.

Eosinophil recruitment and activation in the airway play critical roles in the pathophysiology of allergic asthma (Humbles et al., 2004). Many inflammatory mediators attract and activate eosinophils via signal transduction involving the enzyme PI3K (Li et al., 1999). PI3K activity was stimulated after repetitive OVA challenge, and inhibited by administration of α - and γ -mangostin, leading to suppression of eosinophil recruitment into the airway and lung and it was more effective for reducing airway eosinophils than montelukast, currently available anti-asthmatic drugs. In addition, our data show that administration α - and γ -mangostin significantly reduced in levels of IL-4, IL-5, and IL-13 in BAL fluids. IL-4

and IL-13 are important in directing B cell growth, differentiation, and secretion of IgE. Therefore, the observed reduction in IgE and OVA specific IgE in serum and BAL fluid in OVA-induced allergic asthma by α - and γ -mangostin administration may be the result of inhibitory effects on B cell activation and reduced Th2 cytokine release. Recently, activity of p110 δ catalytic subunit of Class 1B PI3K was reported to be critical for allergen-IgE-induced mast cell degranulation and release of cytokines (Sadhu et al., 2003). Therefore it suggests that administration of α - and γ -mangostin would attenuate not only production of IgE, but also, allergen-IgE-induced mast cell activation during allergic inflammation. Taken together, the observed reduction in airway eosinophilia due to administration of α - and γ -mangostin may be a result of composite effects produced by multiple mechanisms, such as reduction in Th2 cytokines and OVA-specific IgE.

It is believed that inflammation play a critical role in AHR development. Although several Th2 cytokines have been implicated in antigen-induced AHR, IL-13 plays a central role in the development of AHR. IL-13 also activates PI3K and downstream effector molecules (Hershey, 2003). Our data show that administration α - and γ -mangostin significantly inhibits OVA-induced increases in IL-13 levels in BAL fluid and dramatically decreased penh values in response to increasing doses of methacholine. Although recent studies have suggested that there are problems with penh system, it has been widely used as a crude indicator for measuring non-specific bronchial hyperresponsiveness in a mouse model of allergic airway inflammation (Nakaya et al., 2006; Yadav et al., 2011). In addition, it seems that suppressed AHR was affected at least part of inhibitory effects of γ -mangostin on 5-HT_{2A} receptors, which affect airway contraction in vivo and in vitro, and enhances effects of cholinergic nerve-mediated responses.

NF- κ B increases the expression of many genes related to airway inflammation in asthma including inflammatory cytokines, chemokines, adhesion molecules, growth factor, and receptors. NF- κ B activation in airway epithelium also enhances AHR and mucus secretion in OVA-challenged mice (Yang et al., 1998). γ -Mangostin prevents stimulation of NF- κ B, a central mediator of inflammation by LPS, by directly inhibiting IKK activity, which results in a decrease in LPS-induced phosphorylation and degradation of I κ B (Nakatani et al., 2004). Consistent with these observations, we found that γ -mangostin inhibits NF- κ B translocation in OVA-induced allergic asthma mice. α -Mangostin also inhibits NF- κ B translocation. In addition, several studies have shown that PI3K activation enhances NF- κ B signaling through production of phosphatidylinositol 3,4,5-triphosphate, which leads to the stimulation of several downstream targets, including the serine/threonine protein kinase Akt (Ozes et al., 1999). Determination of NF- κ B protein levels in nuclear extracts and p-Akt protein levels in lung tissues in mice treated with α - and γ -mangostin shows that these protein levels were substantially decreased by α - and γ -mangostin, suggesting that reduced PI3K activity successfully inhibits phosphorylation of Akt and NF- κ B activity. These findings suggest that a protective role of α - and γ -mangostin in the pathogenesis of OVA-induced asthma is mediated through an NF- κ B-dependent mechanism.

The phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a well-described negative regulator of the PI3K/Akt signaling pathway, which is one of the most frequently mutated tumor suppressors in human cancer (Yamada and Araki, 2001). Recently, studies have demonstrated that upregulation of PTEN expression or inhibition of PI3K activity leads to the attenuation of allergic airways inflammation and AHR by modulating multiple regulatory pathways in asthma (Ni et al., 2011; Kwak et al., 2003). Thus, we assessed expression of PTEN after α - and γ -mangostin administration. Expression of PTEN protein, which blocks the action of PI3K, was markedly decreased in lungs from OVA-induced

allergic asthma mice, while increased PTEN protein after α - and γ -mangostin administration reduced the allergic airway inflammation.

TGF- β 1 is a multifunctional cytokine that plays key roles in diverse biological processes, including tissue remodeling and repair (Lee et al., 2006). In human chronic lung diseases like asthma, TGF- β 1 expression has been shown to be increased, and the increase in TGF- β 1 expression in asthma patients seems to correlate with disease severity and the degree of peribronchial fibrosis (Minshall et al., 1997). Recently, studies revealed that PI3K and NF- κ B play an important role in regulation of TGF- β 1 expression and tissue fibrosis. Reduced eosinophil recruitment to the airway in PI3K γ -deficient mice challenged with OVA was associated with significantly reduced numbers of TGF- β 1 + peribronchial cells, as well as significantly reduced levels of peribronchial fibrosis (Lim et al., 2009). Therefore, our findings suggest that α - and γ -mangostin may prevent early stage collagen deposition by suppressing TGF- β 1 production.

In conclusion, our results show that α - and γ -mangostin reverse all pathophysiological features of OVA-induced asthma and attenuate the increase in levels of Th2 cytokines, IgE and OVA-specific IgE levels in serum and BALF, and TGF- β 1 in OVA-induced allergic asthma mice, at least in part by the involvement of PI3K/PTEN pathway. The efficacy of α - and γ -mangostin were comparable to that of montelukast and dexamethasone, widely available anti-asthmatic drugs. Hence, α - and γ -mangostin may have therapeutic potential for the treatment of allergic asthma.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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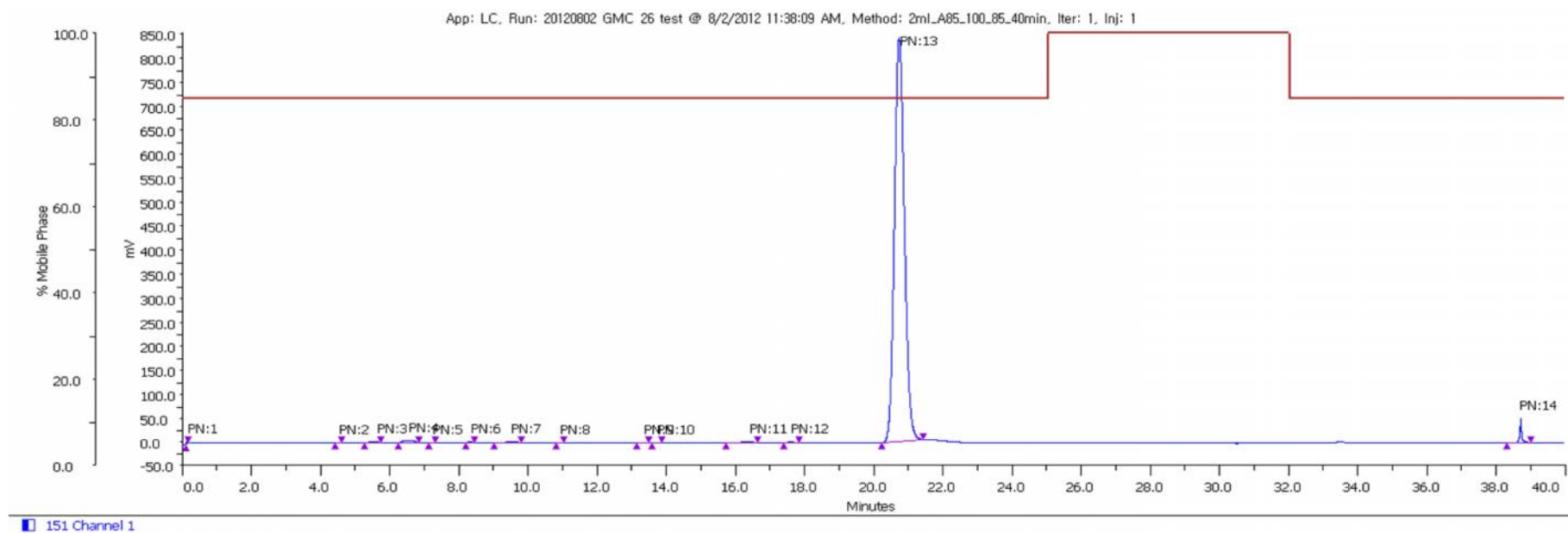
Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fct.2012.08.037>.

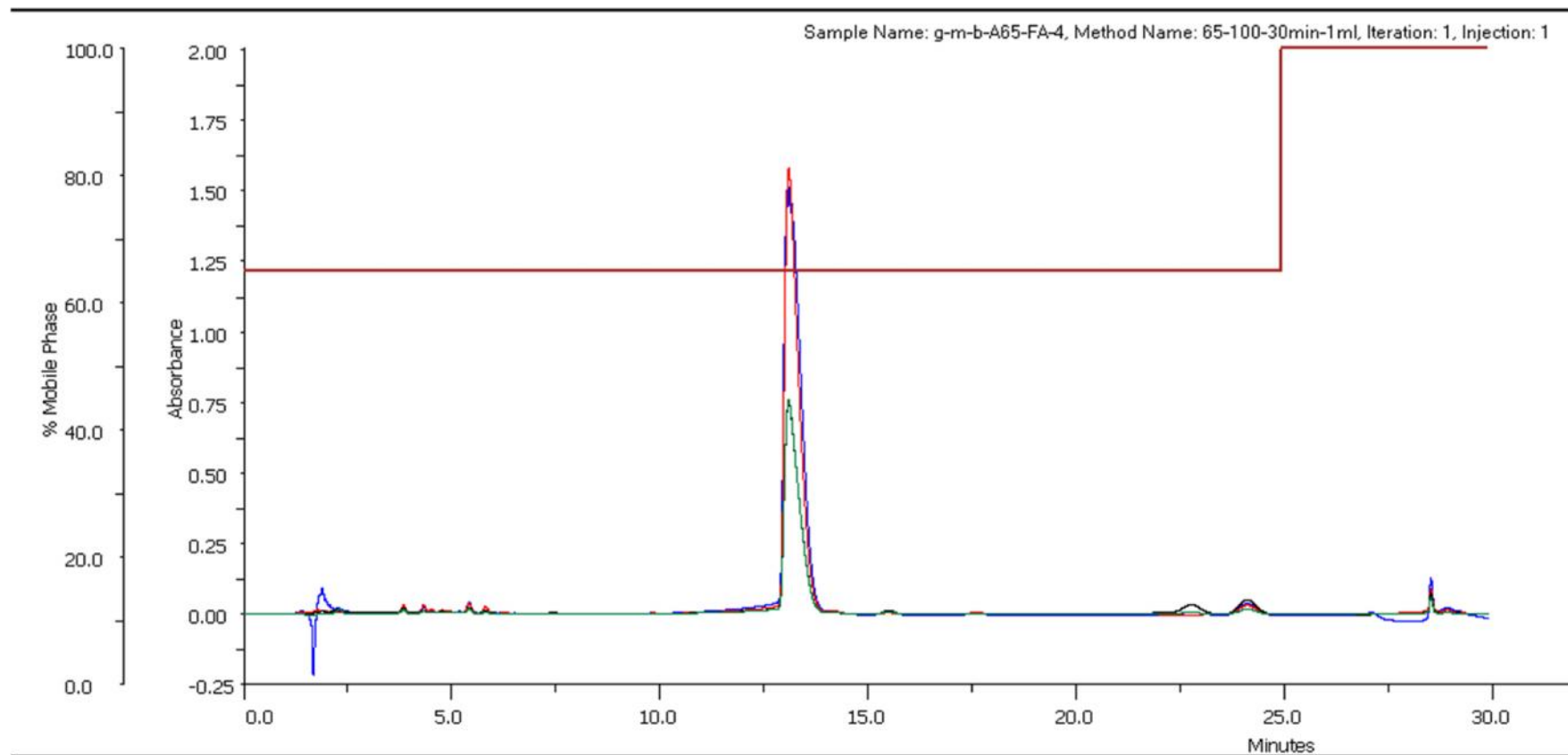
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Alpha mangostin: 85% CAN 2ml/min Luna phenominex semi-prep column
210nm



gamma mangostin: 65% ACN(0.1%formic acid) 1ml/min TSK-Gel
(ODS-80Ts) column 254nm