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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 hyperresponsiveness (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-κB in nuclear protein extracts after OVA challenge. In conclusion, α- and γ-mangostin may have therapeutic potential for the treatment of allergic asthma.

1. Introduction

Allergic asthma is a chronic inflammatory disease characterized by airway infiltration of inflammatory cells inducing airway hyperresponsiveness (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 H_1 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-κB, nuclear factor-kappa B; OVA, ovalbumin; PAS, periodic acid-Schiff; SAL, saline.

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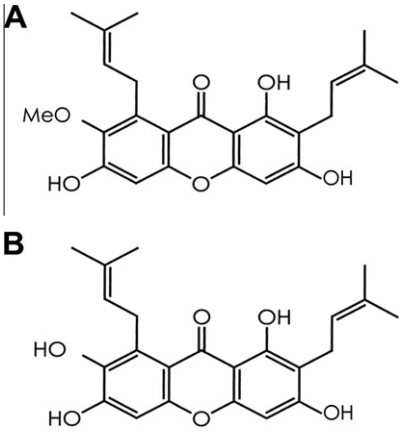

Fig. 1. (A), The chemical structure of α-mangostin; molecular formula, $C_{24}H_{26}O_6$; molecular weight, 410.46. (B), The chemical structure of γ-mangostin; molecular formula, $C_{23}H_{24}O6$; molecular weight, 396.43.

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 percarps 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 μg OVA (Sigma A5503; Sigma–Aldrich, St. Louis, MO, USA), emulsified in 2 mg aluminum hydroxide (Sigma–Aldrich) in a total volume of 200 μl. 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-mangsotin, 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-mangsotin (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.

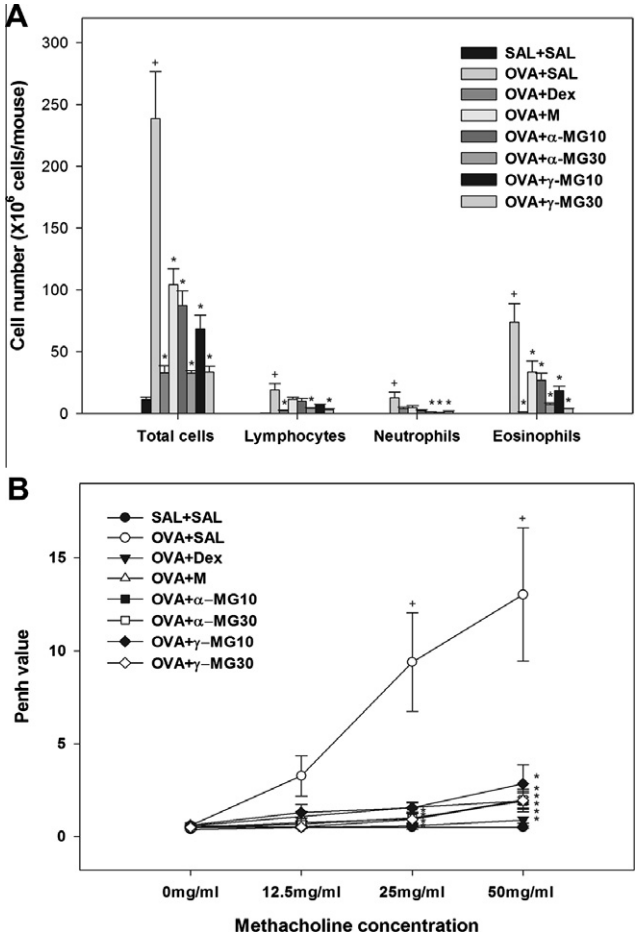

Fig. 2. (A), Effect of α- and γ-mangostin on total cells and differential cellular components in BAL fluids. BAL was performed at 48 h after the last challenge in the following groups: control mice challenged and administered saline (SAL + SAL), OVA challenged mice administered saline (OVA + SAL), OVA 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 ± 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 μm 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.