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Brief Communication

Effect of a (S)-(+)-decursin Derivative, (S)-(+)-3-(3,4-dihydroxy-phenyl)-acrylic Acid 2,2-dimethyl-8-oxo-3,4-dihydro-2H,8H-pyrano[3,2-g]-chromen-3-yl-ester on Apoptosis of Eosinophils and Neutrophils in Normal and Asthmatic Subjects

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(S)-(+)-3(3,4-dihydroxy-phenyl)-acrylic acid 2,2-dimethyl-8-oxo3,4-dihydro-2H,8H-pyrano[3,2-g]Chromen-3-yl-ester (Compound 6, C6) is synthesized from (S)-(+)-decursin and attenuates the pathophysiologic progression of asthma in a ovalbumin-induced asthmatic mouse model. In the present study, we examined the effect of C6 on spontaneous apoptosis of eosinophils and neutrophils of normal and asthmatic subjects. C6 increased the apoptosis of asthmatic eosinophils in a dose-dependent manner, but it inhibited neutrophil apoptosis. C6 has no effect on apoptosis of normal eosinophils and neutrophils. LY294002, an inhibitor of PI3K, rottlerin, an inhibitor of PKCδ, Ro-31-8425, an inhibitor of classical PKC inhibitor, PD98059, an inhibitor of MEK, and BAY 11-7085, an inhibitor of NF-κB, blocked the inhibitory effect on apoptosis of asthmatic neutrophils due to C6. These results indicate that C6 may be valuable as a therapeutic agent for the treatment of asthma.

Key Words: (S)-(+)-decursin derivative, Asthma; Eosinophils, Neutrophils

Asthma is an allergic disease of the airways whose prevalence is increasing yearly (Braman, 2006; Holgate, 2008). Asthma is characterized by airway obstruction, allergen-specific IgE and bronchial inflammation (Busse and Rosenwasser, 2003; Choi et al., 2011). The inflammation is involved in increased infiltration of leukocytes such as eosinophils and neutrophils, and mucus secretion into the airways. Eosinophils and neutrophils function as pathogenic cells in asthma by their release of cytotoxic granule proteins. Constitutive apoptosis in eosinophils and neutrophils is an essential mechanism for their maintenance and persistent accumulation in the asthmatic lung. Number of eosiniophils is inversely propotional to that of neutrophils, depending on asthma severity (Busse and Rosenwasser, 2003; Scheel-Toellner et al., 2004; Jatakanon et al., 1999).

(S)-(+)-decursin is isolated from *Angelica gigas* Nakai (*A. gigas* Nakai) and has been studied for its anti-cancer, anti-bacterial and neuro-protective properties (Jiang C et al., 2006; Lee et al., 2003). Recently, we synthesized (S)-(+)-3(3,4-dihydroxy-phenyl)-acrylic acid 2,2-dimethyl-8-oxo3,4-dihydro-2H,8H-pyrano[3,2-g]Chromen-3-yl-ester (Compound 6, C6) from (S)-(+)-decursin, and demonstrated that C6 suppresses ovalbumin-induced lung inflammation in a mouse model of asthma (Yang et al., 2009). We hypothesized that C6 is associated with apoptosis of eosinophils or neutrophils, and examined if C6 affects the apoptosis of eosinophils and neutrophils of normal and asthmatic subjects.

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Human neutrophils and eosinophils were isolated from heparinized peripheral blood of healthy volunteers and asthmatic subjects using Ficoll-Hypaque gradient centrifugation. Erythrocytes were removed by hypotonic lysis, and then the granulocytes were divided into neutrophils and eosinophils using magnetic cell sorting kit with CD16 microbeads. The CD16-positive cells were neutrophils and the unlabeled cells were eosinophils. The cells were resuspended at 3×10^6/ml in an RPMI 1640 medium with 10% heat-inactivated FBS. Purity of neutrophils and eosinophils was above 97% as assessed by counting the cells by cytospin. This study was approved by the Institutional Review Board of Eulji University for normal volunteers and by the Institutional Review Board of Konyang University for asthma patients. Eosinophils and neutrophils were incubated for 48 h and 24 h in the absence and presence of C6. The

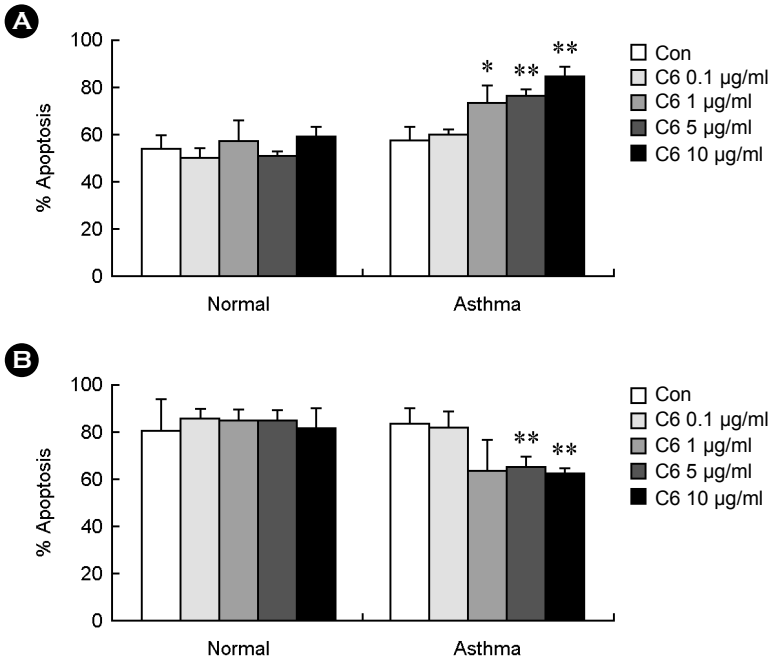

Fig. 1. Efect of C6 on apoptosis of eosinophils and neutrophils of normal and asthmatics. Eosinophils (A) and neutrophils (B) were isolated from human peripheral blood and incubated in the absence (Con) and presence of C6 at the indicated concentration. The apoptosis of these cells were analyzed by measuring the binding of annexin V-FITC and PI using flow cytometry. The percentage of apoptotic cells in total cell population was included all annexin V-binding cells. Data are expressed as the means ± SD. in three individual experiments. *$P < 0.05$ and **$P < 0.01$ indicate a significant difference between the control group and the C6-reated group

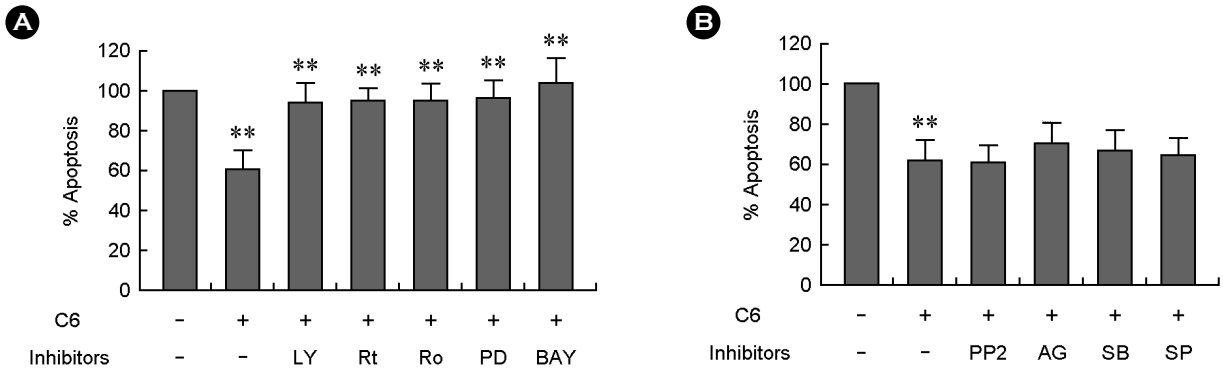

Fig. 2. C6 requires the activation of PI3K, Akt, ERK, and NF-κB for the inhibition of neutrophil apoptosis. Asthmatic blood neutrophils were pre-treated for 1 h with and without 10 μM LY294002 (LY), 10 μM rottlerin (Rt), 10 μM Ro-31-8425 (Ro), 10 μM PD98059 (PD), 10 μM BAY-11-7085 (BAY) (A), 10 μM PP2 (PP2), 10 μM AG490 (AG), 10 μM SB202190 (SB), and 10 μM SP600125 (SP), (B), The cells were incubated for 24 h in the presence and absence of C6 (10 μg/ml). Apoptosis was analyzed by measuring the binding of annexin V-FITC and PI. Data are presented in relation to the control, which was set at 100%. Data are expressed as the means ±SD. **$P < 0.01$ indicates a significant difference between the control group and C6-treated group or between C6-treated group and inhibitor-treated group.

apoptosis of these cells was analyzed using an annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit (BD Biosciences, San Diego, CA) as described previously (Yang et al., 2011). Isolated eosinophils and neutrophils were incubated with an FITC-labeled annexin V and propidium iodide (PI) for 15 min at room temperature. Finally, apoptotic cells were analyzed using a FACSCalibur with CellQuest software (BD bioscience) and were determined as the percentage of cells showing annexin V+/PI- and annexin V+/PI+. Ten thousands events were collected for each experiment.

As shown in Fig. 1, C6 has no effect apoptosis of normal eosinophils and neutrophils. However, C6 altered apoptosis of eosinophils and neutrophils of asthmatic subjects. Apoptotic eosinophils were significantly increased at 48 h after C6 treatment in a dose-dependent manner and apoptotic neutrophils were significantly decreased at 24 h after C6 treatment ($P<0.05$). Corticosteroids such as dexamethasone enhance eosinophil apoptosis but increase neutrophil survival (Druilhe et al., 2003; de Benedictis and Bush, 2012). Although C6 inhibits neutrophil apoptosis, these results indicate that C6 may be a possible drug for treatment of asthma. To investigate the exact mechanism of C6, we examined the alteration of neutrophil apoptosis using signal-specific inhibitors. As shown in Fig. 2, the effect of C6 on neutrophil apoptosis is significantly inhibited by LY294002, an inhibitor of PI3K, rottlerin, an inhibitor of PKCδ, Ro-31-8425, an inhibitor of classical PKC inhibitor, PD98059, an inhibitor of MEK, and BAY 11-7085, an inhibitor of NF-κB ($P<0.01$). PP2, an inhibitor of Src family kinase, AG490, an inhibitor of JAK, SB202190, an inhibitor of p38 MAPK, and SP600125, an inhibitor of JNK have no effect on neutrophil apoptosis altered by C6. These results indicate that C6 transduces its signal via PI3K, PKC, ERK, and NF-κB. Further study on the exact mechanism of C6 may contribute drug development for treatment of asthma.

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