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Anti-asthmatic effects of phenylpropanoid glycosides from *Clerodendron trichotomum* leaves and *Rumex gmelini* herbes in conscious guinea-pigs challenged with aerosolized ovalbumin

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ABSTRACT

Clerodendron trichotomum leaves and *Rumex aquatica* herbs are used as a folk medicine for the treatment of inflammatory diseases, but their active ingredients are not known until now. We isolated caffeic acid and phenylpropanoid glycosides, 1-O-caffeoyl glycoside and acteoside [β -(3',4'-dihydroxyphenyl) ethyl-O- α -L-rhamnopyranosyl(1 $\rightarrow$ 3)- β -D-(4-O-caffeoyl)-glucopyranoside] from their ethylacetate fractions, respectively, and evaluated their anti-asthmatic effects on the aerosolized ovalbumin (OA) challenge in the OA-sensitized guinea-pigs measuring the specific airway resistance (sRaw) during the immediate-phase response (IAR) and late-phase response (LAR), and also measured recruitment of leukocytes and chemical mediators on the bronchoalveolar lavage fluids (BALF) in LAR, as well as histopathological survey. Acteoside and 1-O-caffeoyl glycoside (25 mg/kg) significantly ($P < 0.05$) inhibited sRaw by 32.14 and 26.79% in IAR, and by 55.88% and 52.94% in LAR, respectively, whereas caffeic acid (25 mg/kg) inhibited sRaw by 30.36% in IAR and 44.12% in LAR, compared to control, but with less effective than dexamethasone, disodium cromoglycate, and salbutamol, respectively. In addition, phenylpropanoid glycosides (25 mg/kg) significantly inhibited the recruitments of leukocytes, particularly neutrophils and eosinophils into lung. Furthermore, 1-O-caffeoyl glycoside, acteoside and caffeic acid significantly ($P < 0.05$) inhibited protein content at a dose of 25 mg/kg, and histamine content and PLA₂ activity at a dose of 50 mg/kg, in BALF. Acteoside had more active than caffeic acid and 1-O-caffeoyl glycoside. However, their anti-asthmatic effects were less than the reference drugs. These results indicated that caffeic acid and its glycosides (25 mg/kg) have anti-asthmatic effect as the same manner with dexamethasone and disodium cromoglycate.

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Introduction

Bronchial asthma is a chronic inflammatory disease of the airway that involves multiple components and is orchestrated by numerous cell types, particularly mast cells, eosinophils, and CD4⁺ lymphocytes. Activation of these cells lead to the release of proinflammatory mediators which in turn cause vascular leakage, bronchial smooth muscle contraction, inflammatory cell infiltration, mucous hypersecretion, airway hyperresponsiveness, and ultimately airway remodeling, which are expressed clinically as recurrent wheezing, dyspnea, chest tightness, cough, and air-

way obstruction (Hanania, 2008). Allergen provocation induces an immediate asthmatic response (IAR) and a late-phase asthmatic response (LAR) (Dorsch, 1990; Matsumoto et al., 1994; Khan et al., 2000; Sanjar et al., 1990; De Monchy et al., 1985; Tohda et al., 2000). The specific airway resistance (sRaw) can be observed from 5 to 10 min and at 24 h after antigen challenge in a conscious guinea-pig during the IAR and LAR, which is similar to the segmental antigen challenge in human described by Pepys and Hutchcroft (1975).

Inhaled corticosteroids are recommended for the first-line treatment of persistent disease. However, all patients not achieve asthma control even when these agents are used in high doses and in combination with other medications, including a long-acting β_2 -agonist or a leukotriene modifier (Matsumoto et al., 1994). In addition, prophylactic treatment with drugs such as disodium cromoglycate and glucocorticoids inhibit both the IAR and the LAR, while treatment with a β_2 -selective adrenoceptor agonist such as salbutamol inhibits IAR, but not LAR (Cockcroft and Murdock, 1987; Aryan and John, 1999). However, concern persists regarding the use of inhaled corticosteroids, because the fear is irrational long-term

Abbreviations: BALF, bronchoalveolar lavage fluid; CT, *Clerodendron trichotomum*; IAR, immediate-phase asthmatic response; LAR, late-phase asthmatic response; OA, ovalbumin; PLA, phospholipase A; RG, *Rumex gmelini*; sRaw, specific airway resistance.

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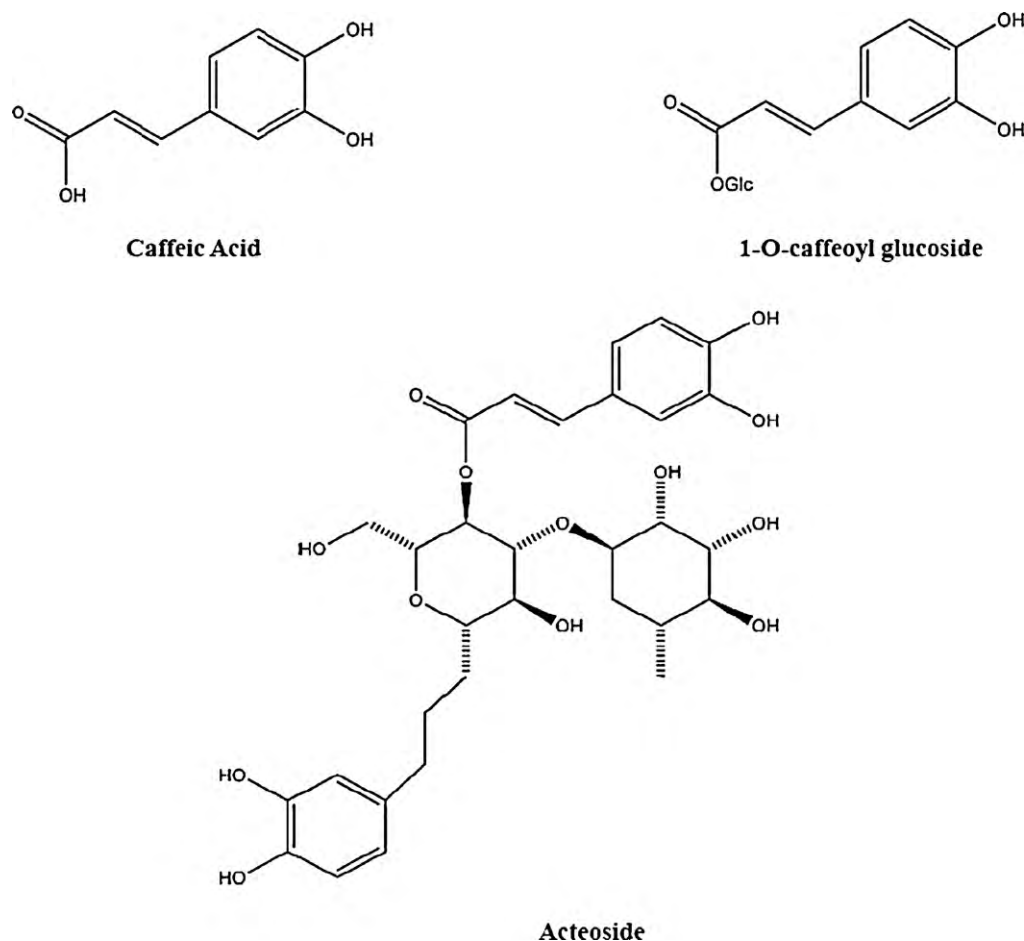


Fig. 1. Chemical structure of phenylpropanoid glycosides.

side effects, such as osteoporosis and growth stunting in children (Barnes, 2006). Although these effective medications are delivered by inhalation, compliance is surprisingly poor (Barnes, 2006; Nauta et al., 2008). In addition, a dichromone flavonoid derivative, disodium cromoglycate, which is a similar chemical structure to the naturally occurring flavonoids, inhibits the release of chemical mediators, and has been used as anti-asthmatic agent, especially the prophylactic treatment of both allergic and exercise-induced asthma via the first dry-powder inhaler (Crompton, 2006). Furthermore, The leaf and stem of *Clerodendron trichotomum* (CT) Thunberg (Family Verbenaceae) have been used a folk medicine for the treatment of inflammatory disease and hypertension in China and Korea (Huang, 1993; Kim et al., 2009; Park and Kim, 2007). We previously isolated anti-inflammatory phenylpropanoid glycosides, acteoside, isoacteoside, decaffeoylacteoside, from CT (Kim et al., 2009; Park and Kim, 2007). In addition, the aerial part of *Rumex gmelini* (RG) Turcz. (Family Polygonaceae) contain anti-inflammatory anthraquinones, stilbenoids and C-glucoside oxanthrones (Wang et al., 2009). However, the biologically active ingredients from them on the chronic inflammatory lung disease, asthma are not known.

We studied effect of caffeic and its glycosides from CT and RG on the asthmatic response by determining the sRaw in the double-chambered plethysmograph during IAR and LAR after challenge of aerosolized-OA in OA-sensitized conscious guinea-pigs (Dorsch and Wagner, 1991; Matsumoto et al., 1994; Sanjar et al., 1990; Jung et al., 2007; Moon et al., 2008), respectively. Furthermore, the recruitment of leukocytes and chemical mediators in bronchoalveolar lavage fluid (BALF) counted as well as histopathological

changes of lung tissue fixed with 10% neutral buffered formalin (NBF) and stained with hematoxylin and eosin (H&E) stain.

Materials and methods

Reagents and medicinal plants

Caffeic acid, ovalbumin (Type V), epiprinhydrinate, dexamethasone, salbutamol, disodium cromoglycate, and Wright's stain were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Thiopental sodium was purchased from Samsung Pharmaceutical Co. (Seoul, Korea). Other reagents used were reagent grade. HEPES-BSS/BSA (pH 7.4) was made by NaCl 132 mM, KCl 6 mM, HEPES 20 mM, KH_2PO_4 1.2 mM, $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ 1.1 mM, MgSO_4 1.0 mM, dextrose 5.5 mM, and bovine serum albumin 1%. Medicinal plants, CT and RG were collected from Naejongsan (Mt.) in July 2001, and were identified by Professor W.K. Whang (Division of Natural Products, College of Pharmacy, Chung-Ang University, Korea). A voucher specimen No. CT-H/001/2001 and RG-AP/001/2001 were deposited in College of Pharmacy, respectively.

Isolation of 1-O-caffeoyl glycoside from RG (Fig. 1)

The fresh aerial parts of RG (5.8 kg) were percolated with methanol (MeOH) and concentrated under vacuum to yield 237.2 g. The MeOH extract was suspended in water and partitioned with ether and water. The water fraction (123 g) was fractionated by Diaion HP-20 (Nippon Rensui Co., Tokyo, Japan) column chromatography eluting with MeOH gradients. 20% MeOH fraction was loaded

on ODS column chromatography eluting with 15% methanol, and obtained caffeic acid and caffeoyl glycoside, 1-O-caffeoyl- β -D-glucopyranoside (2.55 g) which was verified by comparing its IR, MS, and NMR data with literature values (Yoon et al., 2005), and a voucher specimen No. RG-AP001/MeOH005/2002 and RG-AP001/MeOH006/2002 were deposited in College of Pharmacy, Chung-Ang University, respectively.

Isolation of acteoside from CT (Fig. 1)

The dried leaves of CT (600 g) were percolated with 70% MeOH three times, and concentrated under vacuum. The MeOH extract (58 g) was suspended in water and partitioned with ether and water. The water fraction (44.5 g) was fractionated by Diaion HP-20 (Nippon Rensui Co., Tokyo, Japan) column chromatography eluting with MeOH gradients. 80% MeOH fraction was loaded on Sephadex LH-20 and ODS column chromatography eluting with MeOH gradients, and obtained, acteoside, β -(3',4'-dihydroxyphenyl) ethyl-O- α -L-rhamnopyranosyl(1 $\rightarrow$ 3)- β -L-(4-O-caffeoyl)-glucopyranoside (3.4 g) which was verified by comparing its IR, MS, and NMR data with literature values (Kim et al., 2009), and a voucher specimen No. CT-L001/MeOH001/2002 was deposited in College of Pharmacy, Chung-Ang University, Korea.

Animals and sensitization

All animal use procedures were approved by the Institute for Molecular-Based New Drug Development in Chung-Ang University. Male Dunkin–Hartley guinea-pigs (350–400 g) were purchased from Samyuk Animal (Osan, Kyung Ki-Do, Korea), and were housed over 1 week in a normal environmentally controlled animal room (temperature 25 °C, humidity 55%, aeration 10–18/h, illumination 300–500 Lux) with food (Samyang Co., Seoul, Korea) and water *ad libitum*. Animals were actively sensitized to ovalbumin (Sigma, Type V) by two 50 mg injections of the protein given on the same day (i.p. and s.c.), each in 1 ml of 0.9% saline. Twenty-one days after sensitization, 1% OA (0.1 ml/site) was injected intradermally and skin thickness was measured 1 h later. A doubling of skin thickness indicated successful sensitization. Animals that did not respond to intradermal OA were not used (Sanjar et al., 1990).

Challenge and drug treatment

After measuring baseline airway function before drug treatment, the animal was challenged with inhalation of 1% OA or saline for 5 min in the double-chambered plethysmograph (HSE Type 855). Sensitized animals were fasted overnight and treated with drugs or vehicle (saline) 1 h prior to allergen challenge, and again 6, 12 and 23 h later. Thirty minutes before OA challenge, the animals were pretreated with the antihistamine, piprihydinate (5 mg/kg) to prevent sudden death by the systemic hypersensitivity. Twenty-five minutes later, each animal was placed in the double-chambered plethysmograph and permitted to acclimatize for 5 min. The animal was challenged with 10 ml of OA (1.0 mg/ml in saline) or saline aerosol by the jet nebulizer for 5 min (Matsumoto et al., 1994; Jung et al., 2007; Moon et al., 2008).

Measurement of airway function

Airway function in conscious guinea-pigs was measured with the double-chambered plethysmograph (Matsumoto et al., 1994; Sanjar et al., 1990; Jung et al., 2007; Moon et al., 2008; Pennock et al., 1979). Briefly, a conscious animal was positioned with its neck extending through the partition of a head and body chamber. At IAR and LAR, tidal volume, tidal airflow, respiratory rate

and specific airway resistance (sRaw) were measured by volumetric differential pressure transducer (PT5, Grass Co. USA), noninvasive respiratory analyzer, and ink-writing oscilloscope with polygraph (7E, Grass Co., USA), respectively. The phase displacement is measured at the end of inspiration on the steep changeover to the expiration phase. The evaluation unit was linked to a computer for continuous data capture. The increase of sRaw was expressed as a percentage of the baseline value before drug treatment and compared with control (OA): $\text{sRaw increase(\%)} = \{(\text{sRaw after challenge/sRaw before challenge}) - 1\} \times 100$. Furthermore, inhibition in sRaw were expressed as percentage of value of drug-treated group against that of OA-challenged control group using the following equation: $\text{Inhibition of sRaw(\%)} = [1 - (\text{sRaw of drug-treated group/sRaw of OA-challenged control})] \times 100$.

Bronchoalveolar lavage and cell counting

At 24 h after antigen challenge, the animals were terminally anaesthetized with thiopental sodium (30 mg/kg, i.p.) after measurement of lung function parameters, and killed with an overdose of thiopental sodium. The trachea was exposed and cannulated with a polyethylene tube connected to a syringe, and the lungs were lavaged with four 5-ml aliquots of Ca^{2+} and Mg^{2+} free Hank's balanced salt solution (HBSS) containing EDTA (10 mM) and HEPES (10 mM), with all bronchoalveolar lavage fluid (BALF) collected. BALF was centrifuged ($200 \times g$ for 10 min at 4 °C) to remove the supernatant, the cells were resuspended in 1 ml of HBSS, and total leukocytes in BALF counted manually with a hemocytometer. Cell counts were performed on centrifuged preparations (Cytospin II; Shandon Southern Instruments, Pittsburgh, PA) stained with Wright's stain. A minimum of 300 cells were counted and classified as macrophages, eosinophils, neutrophils, lymphocytes, or monocytes based on normal morphologic criteria (Matsumoto et al., 1994; Sanjar et al., 1990; Jung et al., 2007; Moon et al., 2008).

Histamine assay

Histamine in BALF was measured using the fluorometric method (Shore et al., 1959). Briefly, BALF (1 ml) was transferred to a test tube and H_2O (1 ml) added. Four minutes after adding 0.4 ml 1N NaOH followed by 0.1 ml of OPT reagent, 0.2 ml 3N HCl is added. The mixture was thoroughly mixed after each addition, transferred to a 96-well plate, and the fluorescence measured at excitation 350 nm, emission 650 nm with a spectrophotometer (FL600 Microplate Fluorescence Reader, Bio-Tek, USA).

Phospholipase A_2 assay

Using pyrene-labeled phospholipids (10-pyrene PC), PLA_2 activity was measured in the presence of serum albumin with a spectrophotometer at excitation 345 nm, emission 398 nm. Spectrofluorometric analysis of pyrene phospholipids and fatty acids were performed in reaction solution (Radvanyi et al., 1989). The 10-pyrene PC was dried under nitrogen and suspended in ethanol at 0.2 mM. The reaction solution was prepared by adding sequentially 1 ml of buffer containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA; 10 μ l of substrate (2 μ M, final concentration); 10 μ l of a 10% bovine serum albumin solution (0.1%, final concentration) and 6 μ l of 1 M CaCl_2 (6 mM, final concentration). The fluorescence of the reaction medium (blank) was recorded and the reaction was initiated by the addition of BALF. The specific activity in nanomoles per minute and per milligram protein (nmol/min/mg) was obtained by dividing the activity by the amount of protein in milligrams. To calculate the specific activity, protein content was analyzed with a BCA (bicinchoninic acid) protein assay kit (Pierce Co., USA).

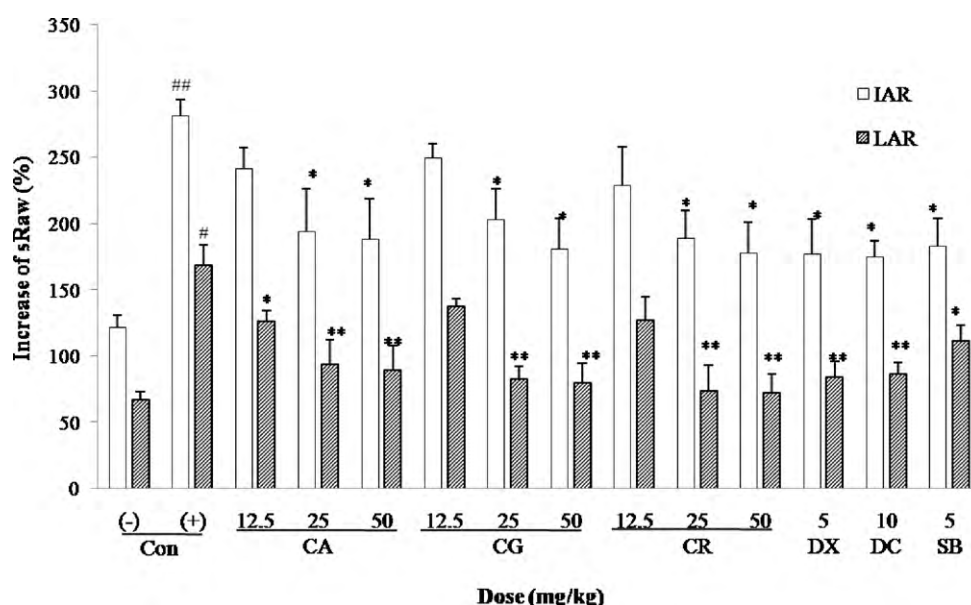


Fig. 2. Inhibitory effects of phenylpropanoid glycosides on sRaw during IAR and LAR. Animals were orally administered test drugs 1 h before, and 6, 12 and 23 h after OA-challenge. CA, caffeic acid; CG, 1-O-caffeoyl glucoside; AS, acteoside; DX, dexamethasone; DC, disodium cromoglycate. Significantly different from negative control (saline), $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ ($n = 6$). Significantly different from control (OA-saline), $^*P < 0.05$, $^{**}P < 0.01$ ($n = 6$).

Histopathological examination

Histologic examination of the lungs was undertaken at the same time points as BAL in animals from each group. Lung tissues were fixed by inflating the lungs with NBF via tracheal cannula to a pressure of 15 cm H₂O and then were removed and stored in NBF at 4°C for histological examination. A length of trachea and two lung slices from similar sites were examined from each animal. The tissues were dehydrated in a graded series of aqueous ethanol solutions and embedded in paraffin. Sections were cut at 3 μ m and were stained with Mayer's H&E before mounting in canada balsam. The predominant eosinophil/neutrophil infiltration into bronchiolar and bronchial tissue was assessed according to method of Richard et al. (1989). The intensity of the "infiltrating" eosinophil response was graded on an arbitrary scale of 0–4 (0 = no response; 1 = mild response, 2 = moderate response; 3 = strong response; 4 = severe response, as judged by the density of leukocytes infiltrating to the peribronchial and peribronchiolar tissues). Leukocytes were negligible in the bronchi and bronchiolar tissues after saline inhalation in antigen-sensitized animals.

Data analysis

All statistical analyses were performed using Sigma-Stat® (Jandel corporation, U.S.A.). An ANOVA test was used to examine the comparisons between the experimental groups and within each test group. All the data is expressed as a mean $\pm$ S.E.M. The comparison between the group means was conducted using a Bonferroni multiple range test with a significance level of $P = 0.05$.

Results

Inhibition of sRaw during IAR and LAR

The mean baseline values of sRaw in saline-challenged guinea-pigs (negative control) and OA-challenged guinea-pigs (positive control) were 2.82 ± 0.11 and 2.73 ± 0.25 mmHg $\times$ s, respectively, but after OA-challenge, the sRaw of negative control and positive control were 6.24 ± 0.49 and 10.40 ± 0.46 mmHg $\times$ s on

IAR, and 4.73 ± 0.38 and 7.33 ± 0.67 mmHg $\times$ s on LAR, respectively. Thus, OA-challenge increased sRaw by 281.0% on IAR and 168.5% on LAR in the positive control, whereas saline-challenge increased sRaw by 121.3% on IAR and 67.7% on LAR in the negative control. These results showed that OA-challenge induced the significant increase of sRaw in both IAR and LAR (Fig. 2).

However, acteoside, 1-O-caffeoyl glycoside and caffeic acid (25 mg/kg) significantly ($P < 0.05$) inhibited sRaw by 32.14, 26.79 and 30.36% in IAR, whereas by 55.88, 52.94 and 44.12% in LAR, compared to that of control group, respectively. Furthermore, dexamethasone (5 mg/kg), disodium cromoglycate (10 mg/kg), and salbutamol (5 mg/kg) significantly ($P < 0.05$) inhibited sRaw by 37.50, 30.21 and 36.07% in IAR, and by 55.88, 52.94 and 38.24% in LAR as compared with that of control, respectively. Acteoside (25 mg/kg) has more inhibitory activity in LAR than dexamethasone (5 mg/kg) and disodium cromoglycate (10 mg/kg) (Fig. 2).

Inhibition of recruitment of leukocytes into BALF

OA-challenge significantly ($P < 0.01$) increased total leukocytes in BALF as compared with negative control group ($7.25 \pm 1.89 \times 10^5$ to $31.28 \pm 6.15 \times 10^5$ cells). OA-challenge significantly ($P < 0.01$) induced the recruitment of eosinophils ($0.50 \pm 0.02 \times 10^5$ to $7.58 \pm 1.45 \times 10^5$ cells), neutrophils ($2.15 \pm 0.87 \times 10^5$ to $7.98 \pm 3.05 \times 10^5$ cells) and macrophages ($0.14 \pm 0.01 \times 10^5$ to $0.66 \pm 0.03 \times 10^5$ cells) in BALF, compared to the negative control group, respectively. These data showed that OA-challenge increased eosinophils by fifteen-fold, and neutrophils and macrophages by over four-fold as compared with that of control group (Fig. 3).

All phenylpropanoid glycosides had inhibited the recruitment of total leukocytes into lung in the dose-dependent manner (Fig. 3). Acteoside and 1-O-caffeoyl glycoside (25 mg/kg) significantly ($P < 0.05$) inhibited leukocytes in BALF as compared with that of control group ($31.25 \pm 6.12 \times 10^5$ to $25.23 \pm 4.72 \times 10^5$ and $26.24 \pm 5.26 \times 10^5$ cells, respectively), whereas caffeic acid (25 mg/kg) significantly ($P < 0.05$) inhibited them ($25.64 \pm 5.34 \times 10^5$ cells). Disodium cromoglycate (10 mg/kg) and dexamethasone acetate (5 mg/kg) significantly

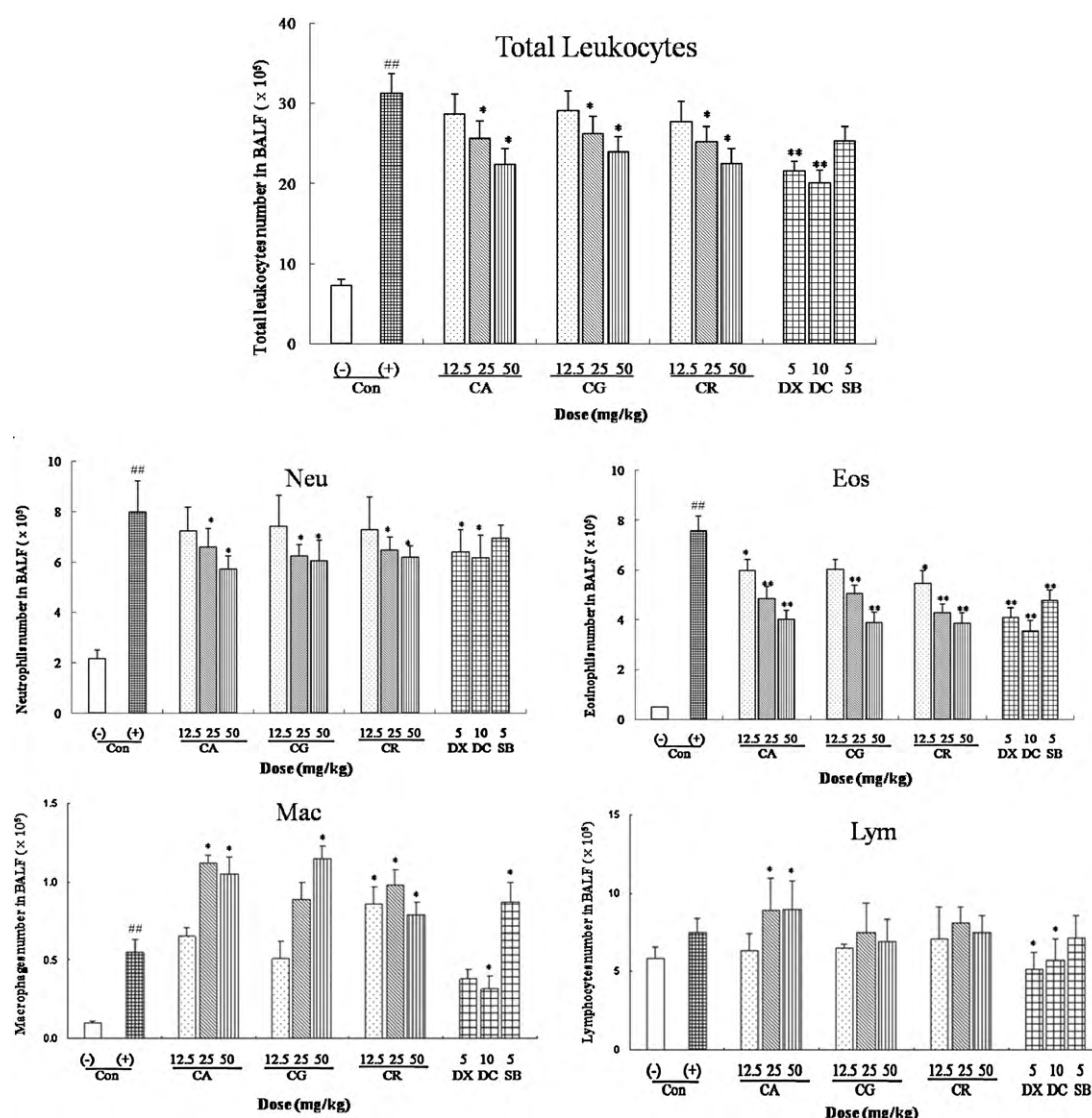


Fig. 3. Inhibitory effects of phenylpropanoid glycosides on the recruitment of total and differential leukocytes in BALF. Animals were orally administered test drugs 1 h before, and 6, 12 and 23 h after OA-challenge. CA, caffeic acid; CG, 1-O-caffeoyl glycoside; AS, acteoside; DX, dexamethasone; DC, disodium cromoglycate. Significantly different from negative control (saline), ^{*} $P < 0.05$, ^{##} $P < 0.01$ ($n = 6$). Significantly different from control (OA-saline), ^{*} $P < 0.05$, ^{**} $P < 0.01$ ($n = 6$).

($P < 0.05$) decreased recruitment of leukocytes in BALF as compared with that of control group, respectively. These results indicated that their inhibitory activities were dependent on the recruitment of eosinophils and neutrophils (Fig. 3).

Improvement of histopathological changes

The histopathological features of lung of saline-challenged guinea-pigs showed normal architecture and any inflammatory cell infiltration (Fig. 4A), whereas aerosolized-OA challenged guinea-pig lungs 24 h after antigen inhalation were seen moderately infiltration of eosinophils and neutrophils in the alveolar sac, the peripheral vasculature and the terminal bronchiole, as well as leukocytes in venules and arterioles, and the detachment of ciliated epithelium (Fig. 4B). However, lung of 1-O-caffeoyl glycoside and acteoside (50 mg/kg)-treated guinea-pigs significantly ($P < 0.05$) inhibited not only the infiltration of inflammatory cell (Fig. 4C and D), particularly, eosinophils and neutrophils in the alveolar sac and the peripheral vasculature by 26.73 and 29.70% (Table 1), respectively, by assessment according to the method of Richard et al.

Inhibition of histamine contents in BALF

OA-challenge of OA-sensitized guinea-pigs significantly ($P < 0.01$) increased the histamine content of BALF compared to the negative control (65.64 ± 10.25 to 334.61 ± 18.32 ng/ml). Thus, histamine released in the BALF of OA-challenge animals increased by 510% during LAR compared to the negative control group. These results showed that histamine release in BALF increased during LAR induced by aerosolized-OA (Fig. 5).

Acteoside, 1-O-caffeoyl glycoside and caffeic acid dosed at 50 mg/kg significantly ($P < 0.05$) decreased the histamine content by 26.40 ± 1.96 , 25.29 ± 2.16 and $19.46 \pm 2.52\%$ in BALF, compared to the OA-challenged control, respectively. In addition, disodium cromoglycate (10 mg/kg) and dexamethasone (5 mg/kg) significantly ($P < 0.05$) inhibited histamine content by 28.12 ± 2.58 and $26.45 \pm 2.38\%$ in BALF, compared to the OA-challenged control, respectively, whereas salbutamol did not inhibit histamine content. These results indicated that their inhibitory effects at 50 mg/kg on histamine release in BALF were nearly similar to that of disodium cromoglycate (10 mg/kg) and dexamethasone (5 mg/kg)-treated groups (Fig. 5).

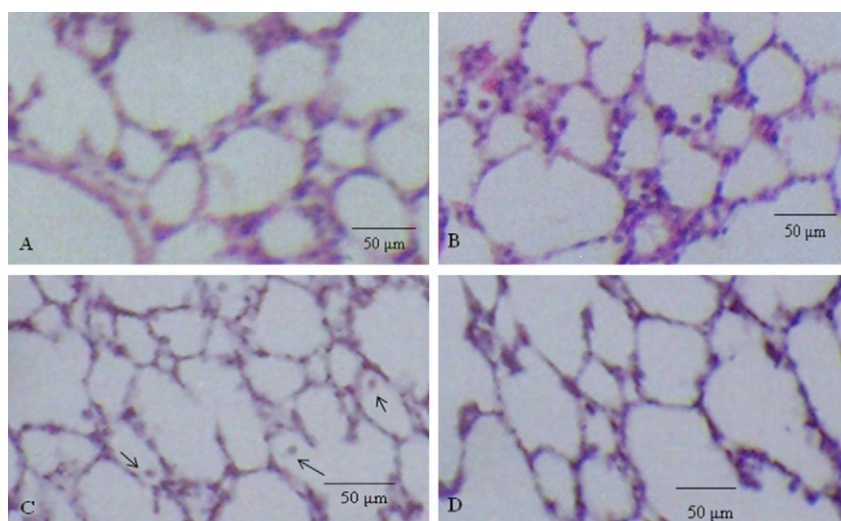


Fig. 4. Representative H&E stained section of lungs taken from OA-sensitized guinea-pigs (A) 24 h after administration of drug vehicle in exposure to saline aerosol, (B) 24 h after administration of drug vehicle and exposure to OA-aerosol, and (C) 24 h after administration of caffeoyl glycoside (50 mg/kg, p.o.), (D) 24 h after administration of acteoside (50 mg/kg, p.o.). Bar indicates 50 μ m. Intraluminal accumulation of eosinophils/neutrophils was noted by the challenge of OA-aerosol, but PGs-treated groups inhibited the intraluminal recruitment of eosinophils/neutrophils (H&E stain; original magnification: $\times 100$).

Inhibition of protein contents in BALF

Protein content in BALF, a marker of inflammatory exudation on the lung in OA-challenged guinea-pigs significantly ($P < 0.05$) was increased by 11-fold compared with saline control (37.82 ± 6.68 to 410.94 ± 17.98 μ g/ml). Acteoside, 1-*O*-caffeoyl glycoside and caffeic acid dosed at 25 mg/kg significantly ($P < 0.05$) decreased the protein content in BALF by 31.86 ± 1.78 , 26.91 ± 2.12 and $23.35 \pm 2.33\%$ in BALF, compared to the OA-inhaled control, respectively. In addition, dexamethasone (5 mg/kg) and disodium cromoglycate (10 mg/kg) significantly ($P < 0.05$) inhibited the protein content by 41.51 ± 3.12 and $39.16 \pm 2.12\%$ in BALF, compared to the positive control, respectively, whereas salbutamol did not inhibit protein content. These results indicated that their inhibitory effects at 50 mg/kg on protein exudation in BALF were less than that of disodium cromoglycate (10 mg/kg)- and dexamethasone (5 mg/kg)-treated groups (Fig. 5).

Inhibition of PLA₂ activity in BALF

PLA₂ activity, a marker enzyme of inflammatory AA metabolite production, on the lung in OA-challenged guinea-pigs significantly ($P < 0.05$) increased by 239.46% (2.99 ± 1.65 to 7.16 ± 0.75 nmol/mg/min) in BALF compared with negative control,

respectively (Fig. 5). 1-*O*-caffeoyl glycoside, acteoside and caffeic acid dosed at 50 mg/kg significantly ($P < 0.05$) decreased PLA₂ activity by 30.17 ± 2.55 , 28.08 ± 2.05 and $26.78 \pm 2.12\%$ in BALF, compared to the OA-challenged control, respectively. In addition, dexamethasone (5 mg/kg) and disodium cromoglycate (10 mg/kg) significantly ($P < 0.05$) inhibited PLA₂ activity by 42.02 ± 3.68 and $28.34 \pm 2.55\%$ in BALF, compared to the positive control, respectively, whereas salbutamol did not inhibit PLA₂ activity. These results indicated that inhibitory effect of caffeoyl glycosides at 25 mg/kg on PLA₂ activity in BALF were less than that of dexamethasone (5 mg/kg)- and disodium cromoglycate (10 mg/kg)-treated groups (Fig. 5).

Discussion

As the our previous work, the model for asthmatic responses in the IAR and the LAR which produced by pretreatment of an anti-histamine, piprinhydrinate (5 mg/kg), without using an adjuvant such as Al(OH)₃ to prevent sudden death due to systemic hypersensitivity, was a conscious, OA-sensitized guinea-pigs (Matsumoto et al., 1994; Sanjar et al., 1990; Jung et al., 2007; Moon et al., 2008; Aoki et al., 2000). In this model, the antigen delivery route is similar to human asthma. After challenge with aerosolized OA of OA-sensitized guinea-pigs, the sRaw increased by over 281.0%

Table 1

Histological assessment of eosinophil infiltration in lung of vehicle-treated and PG-treated guinea-pigs 24 after antigen inhalation.

Drug	Dose (mg/kg)	Infiltration of eosinophil (% inhibition) ^a
Control (OA)	–	3.03 ± 0.21 (–)
Caffeic acid	12.5	2.78 ± 0.18 (8.25)
	25	2.67 ± 0.20 (11.88)
	50	2.43 ± 0.15 (19.02) ^b
	12.5	2.77 ± 0.16 (10.89)
1- <i>O</i> -caffeoyl glycoside	25	2.53 ± 0.19 (16.50)
	50	2.22 ± 0.15 (26.73) ^b
	12.5	2.56 ± 0.21 (15.51)
	25	2.34 ± 0.14 (22.73)
Acteoside	50	2.13 ± 0.15 (29.70) ^b
	5	1.88 ± 0.08 (40.60) ^b
	10	1.92 ± 0.09 (36.63) ^b
Dexamethasone acetate	5	2.93 ± 0.22 (3.30)
Disodium cromoglycate	10	
Salbutamol	5	

^a Graded 0–4 by assessment according to the method of Richard et al. (see “Materials and methods”).

^b $P < 0.05$.

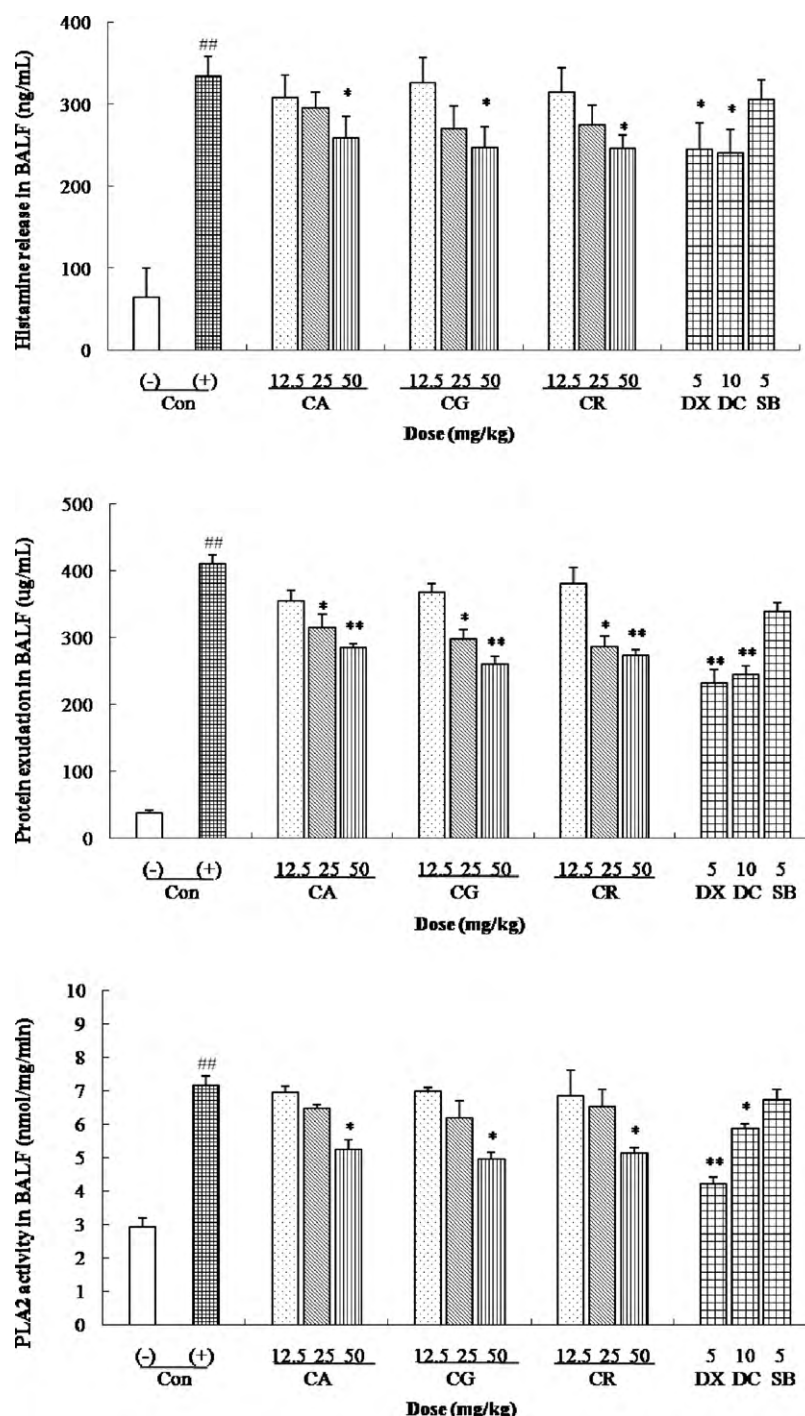


Fig. 5. Inhibitory effects of phenylpropanoid glycosides on protein and histamine contents, and PLA₂ activity in BALF. Animals were orally administered test drugs at 1 h before, and 6, 12 and 20 h after OA-challenge. CA, caffeic acid; CG, 1-O-caffeoyl glucoside; AS, acteoside; DX, dexamethasone; DC, disodium cromoglycate. Significantly different from negative control (saline), **P* < 0.05, ***P* < 0.01 (*n* = 6). Significantly different from control (OA-saline), **P* < 0.05, ***P* < 0.01 (*n* = 6).

during IAR, and over on IAR and 168.5% during the LAR, compared to the saline-challenged control (Fig. 2). In this study, the IAR and LAR were observed 5 min and 24 h after antigen challenge, respectively, similar to the segmental antigen challenge in human described by Kane et al. (1996). Analysis of BALF, and histopathological examinations, showed that leukocytes, particularly eosinophils and neutrophils infiltrated the airway in the guinea-pig asthma model (Figs. 3 and 4, and Table 1), which was similar to the segmental antigen challenge in asthmatic humans with IgE-mediated asthma described by Kane et al. (1996). The infiltrated eosinophils may also release cytotoxic products, such as major basic protein,

eosinophilic cationic protein, and other lipid mediators, including platelet activated factor and cysteinyl-leukotrienes, which lead to tissue damage (De Monchy et al., 1985).

Previously we showed the naturally occurring flavonoids, phenolic compounds and hydroxy pentacyclic triterpene acids had anti-asthmatic effects in the asthmatic murine model (Jung et al., 2007; Moon et al., 2008; Jang et al., 2010; Lee et al., 2010; Cho et al., 2010). Caffeic acid is a selective inhibitor for leukotriene biosynthesis (Koshihara et al., 1984), and caffeic acid phenethyl ester attenuates allergic airway inflammation and hyperresponsiveness in murine model of ovalbumin-induced asthma (Jung et al.,

2008), and inhibits LPS-induced inflammation (Song et al., 2008a,b). In addition, caffeoyl glycoside has immunopotentiality (Zeng et al., 2008), and acteoside inhibits leukocyte accumulation (Hayashi et al., 1994), COX-1 and 2 (Sahpaz et al., 2002), and histamine release (Song et al., 2008a,b). This study additionally indicated caffeic acid and its glycosides from RG and CT significantly inhibited not only the sRaw in IAR and LAR, and also recruitments of eosinophils and neutrophils, and chemical mediators in BALF. Acteoside and 1-O-caffeoyl glycoside had more active than caffeic acid, with less than that of reference drugs. These results indicate that the effects of disodium cromoglycate and dexamethasone on IAR and LAR in this guinea-pig model are similar in human asthma, where salbutamol only can affect sRaw during IAR and LAR, with less effects on the recruitment of chemical mediators during LAR (Matsumoto et al., 1994; Lindmark, 2008). Disodium cromoglycate (10 mg/kg, p.o.) significantly ($P < 0.05$) inhibited sRaw in both IAR and LAR in this asthmatic animal model, whereas absorption of disodium cromoglycate from the gastrointestinal tract is very poor in human and disodium cromoglycate at 2 mg inhaled four times daily has anti-asthmatic effect in human (Svensen et al., 1987). So therefore we believe that the gastrointestinal absorption of disodium cromoglycate in guinea-pig can be different from that in human, and at the low blood level of disodium cromoglycate may inhibit the chemical mediator release from bronchial mast cells and the activation of leukocytes (Svensen et al., 1987). Asthma therapy uses bronchodilator, particularly β -adrenergic stimulants, to reverse immediate-phase airways restriction, and glucocorticoids to reduce lung inflammation (Schuh et al., 2008). Inhalation challenge in asthmatics can induce immediate-phase bronchoconstriction and a subsequent late-phase response associated with edema of the airway wall and lung inflammation. In this asthma model, dexamethasone, disodium cromoglycate and salbutamol significantly inhibited sRaw in IAR and LAR, whereas the β_2 -agonist salbutamol had not inhibited the recruitment of leukocytes and chemical mediators in BALF during LAR. These results are consistent with other Aoki's reports in human asthma, not but in animal asthma (Dahren et al., 1980; Hedqvist et al., 1980; Cockcroft et al., 1987). The inhibitory activity of phenylpropanoid glycosides on sRaw and recruitment of leukocytes were similar with that of dexamethasone in LAR and that of salbutamol in IAR.

In conclusion, these findings indicate that caffeic acid and its glycosides, caffeoyl glycoside (1-O-caffeoyl- β -D-glucopyranoside) from RG, acteoside [β -(3',4'-dihydroxyphenyl)ethyl-O- α -L-rhamnopyranosyl (1 $\rightarrow$ 3)- β -D-(4-O-caffeoyl)-glucopyranoside] from CT dosed at 25 mg/kg significantly inhibited the sRaw during IAR and LAR, in addition, the recruitment of leukocytes, particularly neutrophils and eosinophils, and releases of chemical mediators in BALF.

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