

Anti-asthmatic Action of Quercetin and Rutin in Conscious Guinea-pigs Challenged with Aerosolized Ovalbumin

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The effects of quercetin and rutin on the asthmatic responses were studied in ovalbumin (OA)-sensitized conscious guinea pigs challenged with aerosolized-OA. We measured the specific airway resistance (sRaw) in the double-chambered plethysmograph during the immediate-phase response (IAR) and late-phase response (LAR) at 3~10 min and 24 hr after OA challenge, respectively. We counted leukocytes in bronchoalveolar lavage fluid (BALF) using Wright's stain, as well as in lung tissue fixed with 10% formalin and stained with H & E stain. Quercetin and rutin (7.5 mg/kg, p.o.) significantly and dose-dependently inhibited both sRaw on IAR (31.60 and 26.44%) and LAR (29.87 and 28.69%) but with less efficacy than dexamethasone (3 mg/kg) and salbutamol (0.3 mg/kg), which inhibited IAR by 36.71 and 69.45%, and LAR by 67.23 and 0%, respectively. Quercetin and rutin (15 mg/kg) also inhibited production of histamine, PLA₂, and EPO, and recruitment of leukocytes, particularly neutrophils and eosinophils, during LAR, respectively. Dexamethasone (3 mg/kg) also significantly reduced the recruitment of neutrophils, eosinophils, and lymphocytes in BALF, and salbutamol (0.3 mg/kg) reduced neutrophils and eosinophils with lower activity than dexamethasone. These results indicate that quercetin and rutin may be useful in the treatment of IAR and LAR in asthma via inhibition of histamine release, PLA₂, and EPO, and reduced recruitment of neutrophils and eosinophils into the lung.

Key words: Quercetin, Rutin, Anti-asthmatic action, Double-chambered plethysmograph, Specific airway resistance, Recruitment of neutrophil and eosinophil

Abbreviations: BALF, Bronchoalveolar lavage fluid; BSA, Bovine serum albumin; BSS, Balanced salt solution; EOS, Eosinophils; H & E, Hematoxylin and eosin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IAR, Immediate-phase asthmatic response; IgE, Immunoglobulin E; LAR, late-phase asthmatic response; LLAR, Late late-phase asthmatic responses; OA, Ovalbumin; PLA₂, Phospholipase A₂; PMN, Polymorphonuclear leukocytes; 10-Pyrene PC, Pyrene-labeled phospholipids; sRaw, Specific airway resistance

INTRODUCTION

Allergen-induced bronchial asthma is a chronic inflammatory disease characterized by the presence of inflammatory cells such as lymphocytes, mast cells, and eosinophils in the airways (Bousquet *et al.*, 2000). Allergen provocation induces an immediate asthmatic response (IAR) that peaks at 15 to 30 min and resolves within 2 h, and a late-phase asthmatic response (LAR) which begins 4 to 12 h after allergen challenge (Matsumoto *et al.*, 1994; Glubenkian

et al., 1990; Sanjar *et al.*, 1990; DeMonchy *et al.*, 1985; Wardlaw *et al.*, 1988). Further late responses (late late-phase asthmatic response, LLAR) may occur with diminishing intensity over the subsequent 2 to 3 days (Dunn *et al.*, 1988). Prophylactic treatment with anti-allergic drugs such as cromolyn sodium and glucocorticoids inhibit both IAR and LAR, whereas treatment with β_2 -selective adrenoceptor agonists such as salbutamol inhibits IAR but not LAR (Smith *et al.*, 1991; Cockcroft *et al.*, 1987; Venge *et al.*, 1987). Quercetin, a plant derivative, has anti-asthmatic activity by inhibiting platelet-activating factor (PAF), phospholipase A₂ (PLA₂), and phosphodiesterase (PDE) subtype 3 activity, as well as nitric oxide production, and ovalbumin (OA)-induced bronchial hypersensitivity *in vitro* (Jiang *et al.*, 2006; Ko *et al.*, 2002, 2003, 2004; Griffiths *et al.*, 2002;

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Miller *et al.*, 2001; Dorsh *et al.*, 1991). Plant flavonoids are a large class of polyphenolic compounds with 2-phenyl benzopyrone that have anti-inflammatory (Pettinari *et al.*, 2006; Wu *et al.*, 2004; Li *et al.*, 2001; Skaltasa *et al.*, 2000; Ramesh *et al.*, 1998; Shibata *et al.*, 1991; Gupta *et al.*, 1971; Villar *et al.*, 1984; Fewtrell *et al.*, 1977), anti-allergic (Nishino *et al.*, 1984), anti-oxidant and spasmolytic actions (Omisore *et al.*, 2005; Chakravarthy *et al.*, 1981; Morales *et al.*, 1994).

Quercetin and its glycoside, rutin, are flavonoids, but the effects of these compounds on IAR and LAR of IgE-mediated asthma in conscious OA-sensitized guinea pigs are still unclear. In this study we used this model to measure specific airway resistance (sRaw) in the double-chambered plethysmograph (Matsumoto *et al.*, 1994; Glabenkian *et al.*, 1990; Pennock *et al.* 1979), as well as chemical mediators and leukocytes in bronchoalveolar lavage fluid (BALF), and pathological changes.

MATERIALS AND METHODS

Materials and reagents

Quercetin, rutin, ovalbumin (type V), piprinhydrinate, dexamethasone, salbutamol, and Wright's stain were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 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%.

Animals and sensitization

Male Dunkin-Hartley guinea pigs (350–400 g) were purchased from Samyuk Animal (Osan, Kyung Ki-Do, Korea), and were housed over one 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., 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 (Yeadon *et al.*, 1993). Twenty-one days after sensitization, 1% OA (0.1 mL per 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

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 plethys-

mograph (HSE type 855). Sensitized animals were fasted overnight and treated with drugs or vehicle (saline) 1 h prior to allergen challenge, and again 12 and 20 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).

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). 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. U.S.A.), 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):

$$\text{sRaw increase(\%)} = \frac{\text{sRaw after challenge} - \text{sRaw before challenge}}{\text{sRaw before challenge}} \times 100$$

$$= \{(\text{sRaw after challenge} / \text{sRaw before challenge}) - 1\} \times 100$$

Bronchoalveolar lavage and cell counting

At twenty-four hours 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×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 (Sanjar *et al.*, 1990).

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 H₂O (1 mL) added. Four minutes after adding 0.4 mL 1 N NaOH followed by 0.1 mL of OPT reagent, 0.2 mL 3 N 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, U.S.A.).

Phospholipase A₂ Assay

Using pyrene-labeled phospholipids (10-pyrene PC), PLA₂ 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 CaCl₂ (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., U.S.A.).

Measurement of eosinophil peroxidase (EPO)

The level of free EPO in the supernatant from the BAL fluid was determined as an index of eosinophil activation according to the method described by Strath and co-workers (Strath *et al.*, 1985). An aliquot of BALF was centrifuged at 200 $\times$ *g* for 10 min at 4°C and added to the substrate solution of 0.1 mM *o*-phenylenediamine dihydrochloride (OPD) in 0.05 M Tris-HCl containing Triton X-100 and 1 mM hydrogen peroxide. Except where stated otherwise, all incubations were carried out at pH 8.0 in the presence of 0.1% Triton X-100. One hundred microliter of the substrate was added to 100 μ L of the samples in microtiter plate wells, and the plates were left at 37°C for 30 min before stopping the reaction with 50 μ L 4 M sulfuric acid. The absorbance was measured at 492 nm with a spectrophotometer. Samples containing contaminating hemoglobin in the supernatant were discarded (Banner *et al.*, 1995; Pretolani *et al.*, 1994).

Histological staining

Lung tissues were fixed by inflating the lungs with phosphate-buffered formalin saline via tracheal cannula to a pressure of 15 cmH₂O and were then removed and prepared for staining with hematoxylin and eosin (H & E) before mounting in canada balsam.

Data analysis

All values are presented as mean $\pm$ S.E.M. of 6 animals. Results of airway function measurements are expressed as changes (%) from baseline. Statistical significance was calculated by Student's *t*-test. Differences were considered significant if *P* < 0.05. All statistics were performed with Sigma plot.

RESULTS

Increase of sRaw by OA exposure in OA-sensitized guinea pigs

OA-sensitized guinea pigs had a significantly higher increase in sRaw than naive guinea-pigs in IAR (12.52 ± 1.14 to 29.39 ± 1.38 mmHg $\times$ sec), and LAR (5.68 ± 1.01 to 18.80 ± 1.22 mmHg $\times$ sec), respectively (*P* < 0.01). OA inhalation increased sRaw by $234.74 \pm 4.44\%$ in IAR and $331.00 \pm 9.85\%$ in LAR compared with saline-inhaled control (Fig. 1).

Recruitment of leukocytes into the lung by OA exposure in OA-sensitized guinea pigs

OA-sensitized guinea-pigs in LAR after OA challenge had significantly (*P* < 0.01) more total leukocytes in BALF compared with naive animals ($19.06 \pm 3.21 \times 10^6$ to 63.44

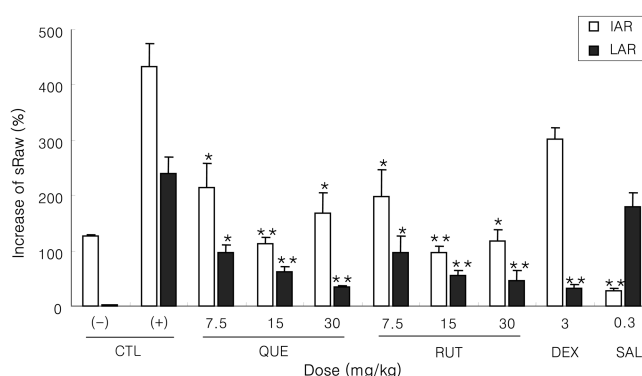


Fig. 1. Flavonoids inhibit sRaw in IAR and LAR. Drugs were orally administered 1 h prior to OA challenge, and again 12 and 20 h later. sRaw increased in IAR and LAR at 3–10 min and 24 h after allergen challenge, respectively. Increase of sRaw (%) = {(sRaw after challenge/sRaw before challenge) - 1} $\times$ 100. Each value represents the mean $\pm$ S.E.M (n=6). Significantly different from OA-inhaled control, *, *P* < 0.05 and **, *P* < 0.01. (-) CTL; saline-inhaled control, (+) CTL; OA-inhaled control, QUE; quercetin, RUT; rutin, DEX; dexamethasone, SAL; salbutamol.

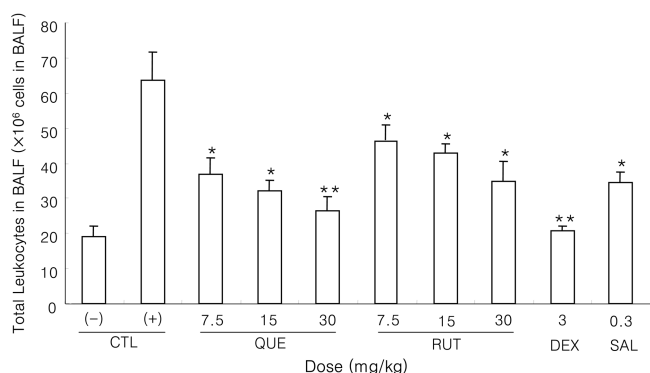


Fig. 2. Effects of flavonoids on total leukocytes in BALF in LAR. Drugs were orally administered 1 h prior to OA challenge, and again 12 and 20 h later. Each value represents the mean $\pm$ S.E.M (n=6). Significantly different from OA-inhaled control, *, $P < 0.05$ and **, $P < 0.01$. Abbreviations are the same as Fig. 1.

$\pm 8.17 \times 10^6$ cells) (Fig. 2). The ovalbumin challenge caused leukocytosis, with neutrophils and eosinophils in BALF increasing significantly by 10 and 5.4 times ($1.40 \pm 0.23 \times 10^6$ to $14.59 \pm 4.19 \times 10^6$ and $3.24 \pm 0.68 \times 10^6$ to $17.63 \pm 2.90 \times 10^6$ cells), respectively (Fig. 3).

OA Exposure increases histamine, protein, PLA₂, and EPO in BALF

Histamine contents in BALF of naive and OA-sensitized

guinea-pigs were 125.00 ± 7.18 and 396.00 ± 32.02 ng/mL on LAR, respectively, or an increase of 316.80 ± 44.21 % in sensitized guinea-pigs. Protein levels, a measure of exudates, in naive and sensitized animals were 101.3 ± 16.8 and 409.8 ± 25.3 μ g/mL on LAR, respectively (an increase of 404.54 ± 24.98 %). Phospholipase A₂ (PLA₂) activity in BALF of naive and sensitized guinea-pigs was 2.97 ± 0.20 and 7.40 ± 0.31 nmol/min/mg on LAR, respectively (an increase of 249.16 ± 15.50 %). EPO, a marker of eosinophil activation, in naive and sensitized guinea-pigs was OD (optical density) 0.29 ± 0.07 and 5.31 ± 0.43 on LAR, respectively (an increase of 1831.03 ± 61.48 %) (Fig. 4).

Histopathological changes by OA exposure in OA-sensitized guinea pigs

The lungs of sensitized guinea pigs showed recruitment of eosinophils and neutrophils in the alveolar sac, peripheral vasculature and terminal bronchiole, the pavement of leukocytes in venule and arteriole, and detachment of ciliated epithelium (Fig. 5(B)), with only a few inflammatory cells around the terminal bronchioles in normal guinea pigs (Fig. 5(A)), as in other reports (Toward *et al.*, 2004; Yeadon *et al.*, 1993; Sanjar *et al.*, 1990; Richard *et al.*, 1989).

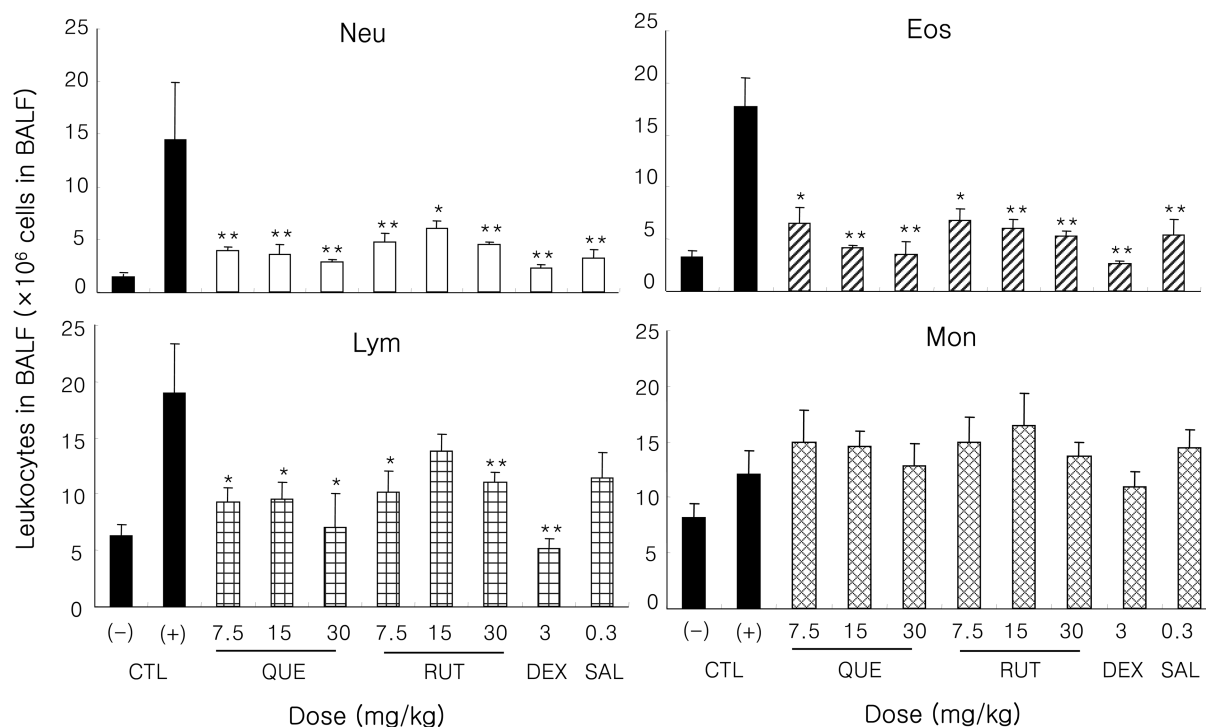


Fig. 3. Flavonoids inhibit recruitment of eosinophils, neutrophils, lymphocytes and monocytes in BALF in LAR. Drugs were orally administered 1 h prior to OA challenge, and again 12 and 20 h later. Inhibition (%) = $(a-c)/(a-b) \times 100$, a and b; saline- and OA-inhaled control, c; test drug-treated group. Each value represents the mean $\pm$ S.E.M (n=6). Significantly different from OA-inhaled control, *, $P < 0.05$ and **, $P < 0.01$. Abbreviations are the same as Fig. 1.

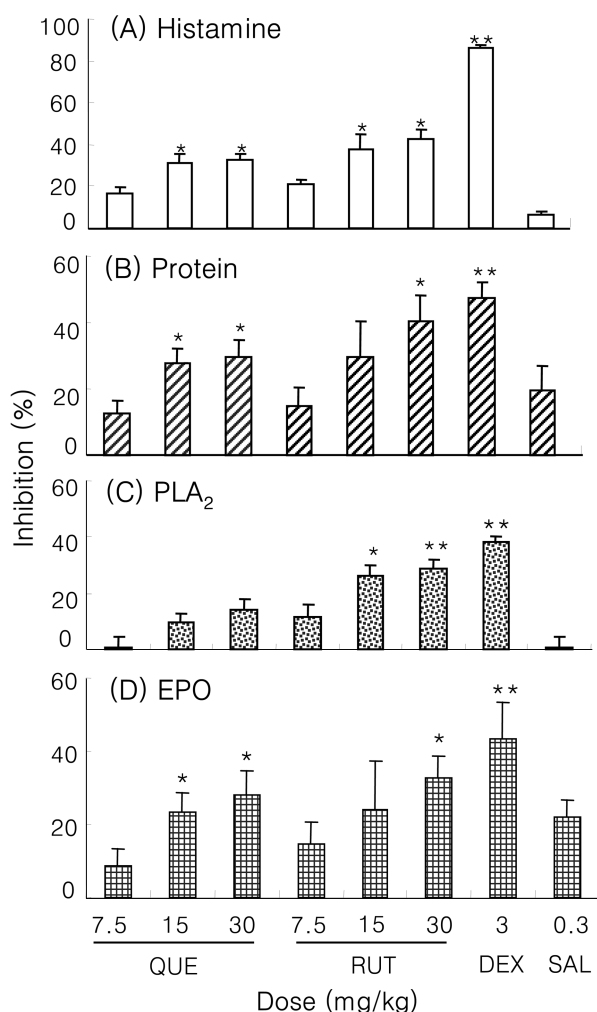


Fig. 4. Flavonoids inhibit histamine release, protein exudation, PLA₂ and EPO activity in BALF in LAR. Drugs were orally administered 1 h prior to OA challenge, and again 12 and 20 h later. Inhibition (%)=(a-c)/(a-b)×100, a and b; saline- and OA-inhaled control, c; test drug-treated group. Each value represents the mean ± S.E.M (n=6). Significantly different from OA-inhaled control, *, P< 0.05 and **, P< 0.01. Abbreviations are the same as Fig. 1.

Effect of quercetin and rutin on OA-induced IAR and LAR

Decrease of sRaw

Quercetin (7.5 mg/kg, p.o.) significantly decreased ($P<0.01$) sRaw by 29.87 ± 4.11 in IAR and $31.64 \pm 8.93\%$ in LAR compared with control (29.39 ± 1.38 to 20.61 ± 2.11 and 18.80 ± 1.22 to 12.86 ± 2.42 mmHg×sec, respectively) (Fig. 1). Rutin (7.5 mg/kg, p.o.) also decreased significantly ($P<0.01$) sRaw in IAR and LAR by 28.72 ± 7.25 and $26.44 \pm 6.17\%$ compared with control (29.39 ± 1.38 to 20.95 ± 3.73 and 18.80 ± 1.22 to 13.83 ± 0.85 mmHg×sec, respectively) (Fig. 1). Dexamethasone (3 mg/kg) significantly decreased sRaw in IAR and LAR by 36.71 ± 3.16 and $67.23 \pm 4.04\%$, respectively ($P<0.01$). Salbutamol (0.3

mg/kg) significantly decreased sRaw in IAR by $69.45 \pm 5.38\%$ ($P<0.01$), but not in LAR (Fig. 1). Thus, flavonoids act as well as dexamethasone in both IAR and LAR.

Decreased recruitment of total leukocytes in BALF

Quercetin and rutin dose-dependently inhibited total leukocyte accumulation in BALF ($P<0.05$). Quercetin and rutin (7.5 mg/kg) significantly inhibited recruitment of leukocyte in BALF by $37.45 \pm 3.48 \times 10^6$ and $45.50 \pm 4.35 \times 10^6$ cells, respectively ($P<0.05$) (Fig. 2). Dexamethasone (3 mg/kg) and salbutamol (0.3 mg/kg) also significantly inhibited total leukocytes (Fig. 2).

Decreased recruitment of neutrophils, eosinophils, and lymphocytes in BALF

Quercetin and rutin (7.5 mg/kg) significantly inhibited recruitment of neutrophils, eosinophils, and lymphocytes in BALF (Fig. 3). Dexamethasone (3 mg/kg) also significantly decreased the recruitment of neutrophils, eosinophils and lymphocytes in BALF, as did salbutamol (0.3 mg/kg), but with less efficacy (Fig. 3).

Decrease of the chemical mediators in BALF

Flavonoids decreased chemical mediators such as histamine and protein contents, and PLA₂ and EPO activity in BALF compared with control in a dose-dependent manners (Fig. 4). Rutin (15 mg/kg) significantly decreased histamine release ($39.48 \pm 3.37\%$, $P<0.05$) and PLA₂ specific activity ($27.35 \pm 1.56\%$, $P<0.05$) in BALF compared with control. Quercetin (15 mg/kg) also significantly decreased histamine ($31.72 \pm 2.52\%$, $P<0.05$), protein exudation ($28.97 \pm 2.14\%$, $P<0.05$), and EPO activity ($21.67 \pm 1.78\%$, $P<0.05$) in BALF compared with control. Dexamethasone (3 mg/kg) significantly ($P<0.01$) inhibited histamine release, protein exudation, EPO and PLA₂ specific activity, but salbutamol (0.3 mg/kg) slightly decreased all of them compared with control (Fig. 4).

Improvement of histopathological changes

Quercetin and rutin (15 mg/kg) inhibited the infiltration of inflammatory cells, particularly 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. 5).

DISCUSSION

Here, we developed an asthma model of IAR and LAR in guinea pigs as described by other reports (Yeadon *et al.*, 1993; Huston *et al.*, 1988). Guinea-pigs readily show airway hyper-reactivity and eosinophilia (Toward *et al.*, 2004; Yeadon *et al.*, 1993; Sanjar *et al.*, 1990; Dunn *et al.*,

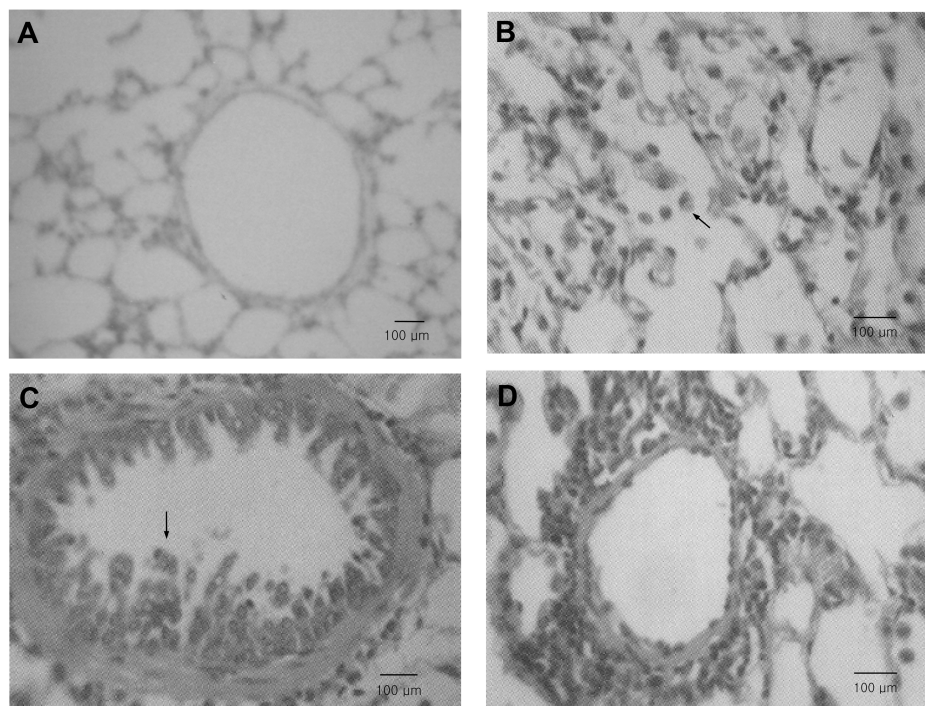


Fig. 5. (A) Lung of naive control 24 h after exposure of saline aerosol. Slight inflammatory cell infiltration around terminal bronchiole with normal structural architecture. (B) Lung of OA-control 24 h after exposure of aerosolized-OA. Recruitment of leukocytes, particularly eosinophils, and narrowing of alveolar sacs (arrow). (C) Lung of quercetin (15 mg/kg)-treated group 24 h after exposure of aerosolized-OA. Detachment of ciliated epithelium in secondary bronchioles and infiltration of leukocytes around bronchiole (arrow), but less recruitment of leukocytes in alveolar sacs. (D) Lung of rutin (15 mg/kg)-treated groups 24 h after exposure of aerosolized-OA. Infiltration of leukocytes in narrowed alveolar sacs, with leukocytes present in arterioles, but all pathologic changes improved compared with control (OA) (H & E, $\times 400$).

1988; Huston *et al.*, 1988; Kallos *et al.*, 1984). Guinea-pigs were challenged by inhalation of aerosolized antigen, a similar delivery route in human asthma (Aoki *et al.*, 1988; Andersson *et al.*, 1988; Abraham *et al.*, 1988; Turner *et al.*, 1990) and lung segmental antigen challenge in human asthma (Kane *et al.*, 1996; Kane *et al.*, 1996). In this experiment, aerosolized-OA inhalation increased sRaw in IAR and LAR by 2.3 and 3.3 times compared with naive control, and increased neutrophil and eosinophil in BALF by 10 and 5.4 times compared with control. BAL technique and subsequent histopathological studies showed the recruitment of leukocytes, particularly eosinophils and neutrophils, into the lung by 5.4 and 9.4 times, similar to a segmental challenge in human lung (Kane *et al.*, 1994). Pre-treatment of the anti-histamine, piprinhydrinate prevented immediate anaphylaxis for the longer LAR model. Thus, aerosolized-OA inhalation in guinea pigs can induce asthma as in humans.

The effects of salbutamol and dexamethasone on IAR and LAR in this guinea-pig model are similar in human asthma, where salbutamol affects IAR not but LAR, and dexamethasone can affect LAR, with less effects on IAR. Quercetin and rutin significantly inhibited sRaw and the recruitment of leukocytes, with rutin less active than quer-

cetin. We also showed that these flavonoids have anti-inflammatory and anti-allergy activity because of inhibition of mast cell degranulation and histamine release (Kim *et al.*, 1991; Kim *et al.*, 1990), and other glycosylated derivatives of flavonoids are less effective than their corresponding aglycones, e.g. rutin vs quercetin or phloretin vs phlorizin (Havsteen *et al.*, 1983). Bacterial glycosidase can liberate the flavonoid aglycone (Middleton *et al.*, 1984). Oral flavonoids are largely present as aglycones in the intestine and absorbed as micelles into the blood (Middleton *et al.*, 1982) since they have better lipid solubility (Bennett *et al.*, 1981). The glycosidic groups stereologically hinder a portion of the molecule necessary for inhibitory activity (Middleton *et al.*, 1984).

Asthma therapy uses bronchodilators, particularly β -adrenergic stimulants, to reverse immediate-phase airway restriction, and glucocorticoids to reduce lung inflammation (Sanjar *et al.*, 1990). 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 significantly inhibited sRaw in LAR not but IAR. These results are consistent with Matsumoto's report (1994) and other reports in human

asthma, not but in animal asthma (Dahren *et al.*, 1980; Hedqvist *et al.*, 1980; Marie *et al.*, 1955). The β_2 -agonist salbutamol inhibited only IAR (Matsumoto *et al.*, 1994).

Accumulation of inflammatory cells in the lung is a pathological feature of asthmatics (Dahren *et al.*, 1980), and recruitment of leukocytes, particularly of eosinophils, in the late response to allergen inhalation is characteristic of the late response to allergen inhalation in man (DeMonchy *et al.*, 1985; Middleton *et al.*, 1982). Kim (1993) reported that leukotriene B₄ is a chemoattractant of eosinophils into the lung (Bennett *et al.*, 1981). Flavonoids inhibit phospholipid metabolism. Leukotrienes are derived from arachidonic acid through 5-LO and then nucleophilic attack to produce peptidoleukotrienes. These 5-LO products mediate constriction of airway smooth muscle, leukocyte chemotaxis (Dahren *et al.*, 1980; Hedqvist *et al.*, 1980) and vascular permeability (Kim *et al.*, 1996; Middleton *et al.*, 1982, 1984; Havsteen *et al.*, 1983; Bennett *et al.*, 1981; Krell *et al.*, 1981; Dahren *et al.*, 1980; Hedqvist *et al.*, 1980; Drazen *et al.*, 1980; Holryde *et al.*, 1970; Pichurko *et al.*, 1989; Ford-Hutchison *et al.*, 1980). Therefore, inhibiting 5-LO can attenuate leukotriene production. It is nuclear whether quercetin inhibited 5-LO to block eosinophil accumulation. Eosinophils play an important role in the pathogenesis of airway hyperresponsiveness (Kazuhiro *et al.*, 1993; Durham *et al.*, 1985), and pulmonary eosinophilia can be caused by epithelial damage, decreased mucociliary clearance, nonspecific hyperreactivity, bronchial smooth muscle hyperreactivity, and bronchial smooth muscle hyperplasia. Results of the present study reveal that pre-treatment of dexamethasone inhibited the pulmonary hypersensitivity and eosinophil accumulation, as well as dexamethasone inhibited recruitment of leukocytes, but salbutamol did not act as well as dexamethasone. In conclusion, we found that quercetin and rutin dose-dependently reduced increase of the sRaw and eosinophil accumulation in asthma. These findings indicate that quercetin and rutin can be useful may have therapeutic potential for asthma in clinical practice.

Quercetin and rutin dose-dependently inhibited sRaw in LAR and IAR, as well as the recruitment of leukocytes, particularly eosinophils and neutrophils on LAR, with quercetin showing more activity than rutin. Dexamethasone also significantly inhibited sRaw and leukocyte recruitment. Salbutamol significantly inhibited sRaw in IAR. Quercetin and rutin inhibited sRaw and leukocyte recruitment at a similar level as dexamethasone in LAR and salbutamol in IAR. Thus, quercetin and rutin may be useful in the treatment of IAR and LAR in asthma.

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REFERECES

- Abraham, W. M., Sielczak, M. W., Wanner, A., Perruchoud, A. P., Blinder, L., Stevenson, J.S., Ahmed, A., and Yerger, L.D., Cellular markers of inflammation in the airways of allergic sheep with and without allergen-induced late responses. *Am. Rev. Respir. Dis.*, 138, 1565-1571 (1988).
- Andersson, P., Brange, C., von Kogeler, B., Sonmark, B., and Stahre, G., Effect of glucocorticosteroid treatment on ovalbumin-induced IgE-mediated immediate and late allergic response in guinea pig. *Int. Arch. Allergy Appl. Immunol.*, 87, 32-39 (1993).
- Aoki, S., Boubekeur, K., Kristersson, A., Morley, J., and Sanjar, S., Is allergic airway hyperreactivity of the guinea pig dependent on eosinophil accumulation in the lung? *Br. J. Pharmacol.*, 94, 365-371 (1988).
- Bennett, J. P., Gomperts, B. D., and Wollenweber, E., Inhibitory effects of natural flavonoids on secretion from mast cells and neutrophils. *Arzneimittelforschung.*, 31, 433-437 (1981).
- Bousquet, J., Jeffery, P., Busse, W. W., Johnson, M., and Vignola, A. M., Asthma: from bronchoconstriction to airway inflammation and remodeling. *Am. J. Respir. Care Med.*, 161, 1720-1745 (2000).
- Chabot-Fletcher, M. C., Underwood, D. C., Breton, J. J., Adams, J. L., Kagey-Sobotka, A., Griswold, D. E., Marshall, L. A., Sarau, H. M., Winkler, J. D., and Hay, D. W., Pharmacological characterization of SB 202235, a potent and selective 5-lipoxygenase inhibitor: effects in models of allergic asthma. *J. Pharmacol. Exp. Ther.*, 273, 1147-1155 (1995).
- Chakravarthy, B. K., Rao, V. Y., Gambhir, S. S., and Gode, K. D., Isolation of Amentoflavone from *Selaginella rupestris* and its Pharmacological Activity on Central Nervous System, Smooth Muscles and Isolated Frog Heart Preparations. *Planta Med.*, 43, 64-70 (1981).
- Cockcroft, D. W. and Murdok, K. Y., Comparative effect of inhaled salbutamol, sodium cromoglycate and beclomethasone dipropionate on allergen-induced early asthmatic responses, late asthmatic responses and increased bronchial hyperreactivity to histamine. *J. Allergy Clin. Immunol.*, 79, 734-740 (1987).
- Dahren, S. E., Bjork, J., Hedqvist, P., Hammarstorm, S., and Samuelsson, B., Leukotriens are potent constrictors of human bronchi. *Nature*, 288, 484-486 (1980).
- DeMonchy, J. G. R., Kauffman, H. F., and Venge, P., Bronchoalveolar eosinophilia during allergen induced late asthmatic reactions. *Am. Rev. Respir. Dis.*, 131, 373-376 (1985).
- Dorsch, W., Ettl, M., Hein, G., Scheftner, P., Weber, J., Bayer, T., and Wagner, H., Antiasthmatic effects of onions. Inhibition of platelet-activating factor-induced bronchial obstruction by onion oils. *Int. Arch. Allergy Appl. Immunol.*, 82, 535-536 (1987).

- Dorsch, W. and Wagner, H., New antiasthmatic drugs from traditional medicine? *Int. Arch. Allergy Appl. Immunol.*, 94, 262-265 (1991).
- Drazen, J. M., Austen, K. F., Lewis, R. A., Clark, D. A., Goto, G., Marfat, A., and Corey, E. J., Comparative airway and vascular activities of leukotrienes C-1 and D *in vivo* and *in vitro*. *Proc. Natl. Acad. Sci. U S A.*, 77, 4354-4358 (1980).
- Dunn, C. J., Elliot, G. A., Oosteen, J. A., and Richards, I. M., Development of a prolonged eosinophil-rich inflammatory leukocyte infiltration in the guinea-pigs asthmatic response to ovalbumin inhalation. *Am. Rev. Respir. Dis.*, 137, 541-547 (1998).
- Fewtrell, C. M. S. and Gomperts, B. D., Effect of flavone inhibitors of transport ATPases on histamine secretion from rat mast cells. *Nature*, 265, 635-636 (1997).
- Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E., and Smith, M. J., Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature*, 286, 264-265 (1980).
- Griffiths, G., Trueman, L., Crowther, T., Thomas, B., and Smith, B., Onions--a global benefit to health. *Phytother. Res.*, 16, 603-615 (2002).
- Gulbenkian, A. R., Fernandez, X., Kreutner, W., Minnicozzi, M., Watnick, A. S., Kung, T., and Egan, R. W., Anaphylactic challenge causes eosinophil accumulation in bronchoalveolar lavage fluid of guinea pigs. *Am. Rev. Respir. Dis.*, 142, 680-685 (1990).
- Gupta, M. B., Bhallar, T. N., Gupta, G. P., and Bhargava, K. P., Anti-inflammatory activity of taxifolin. *Jpn. J. Pharmacol.*, 21, 377-382 (1971).
- Havsteen, B., Flavonoids, a class of natural products of high pharmacological potency. *Biochem. Pharmacol.*, 32, 1141-1148 (1983).
- Hedqvist, P., Dahlén, S. E., Gustafsson, L., Hammarström, S., and Samuelsson, B., Biological profile of leukotrienes C₄ and D₄. *Acta Physiol. Scand.*, 110, 331-333 (1980).
- Holroyde, M. C., Altounyan, R. E., Cole, M., Dixon, M., and Elliott, E. V., Bronchoconstriction produced in man by leukotrienes C and D. *Lancet.*, 2, 17-18 (1981).
- Huston, P. A., Church, M. K., Clay, T. P., Miller, P., and Holgate, S. P., Early and late-phase bronchoconstriction after allergen challenge of nonanesthetized guinea pigs. I. The association of disordered airway physiology to leukocyte infiltration. *Am. Rev. Respir. Dis.*, 137, 548-557 (1988).
- Hutson, P. A., Holgate, S. T., and Church, M. K., The effect of cromolyn sodium and albuterol on early and late phase bronchoconstriction and airway leukocyte infiltration after allergen challenge of nonanesthetized guinea pigs. *Am. Rev. Respir. Dis.*, 138, 1157-1163 (1988).
- Jiang, J. S., Shih, C. M., Wang, S. H., Chen, T. T., Lin, C. N., and Ko, W. C., Mechanisms of suppression of nitric oxide production by 3-O-methylquercetin in RAW 264.7 cells. *J. Ethnopharmacol.*, 103, 281-287 (2006).
- Kallós, P. and Kallós, L., Experimental asthma in guinea pigs revisited. *Int. Arch. Allergy Appl. Immunol.*, 73, 77-85 (1984).
- Kane, G. C., Pollice, M., Kim, C. J., Cohn, J., Dworski, R. T., Murray, J. J., Sheller, J. R., Fish, J. E., and Peters, S. P., A controlled trial of the effect of the 5-lipoxygenase inhibitor, zileuton, on lung inflammation produced by segmental antigen challenge in human beings. *J. Allergy Clin. Immunol.*, 97, 646-654 (1996).
- Kane, G. C., Tollino, M., Pollice, M., Kim, C. J., Cohn, J., Murray, J. J., Dworski, R., Sheller, J., Fish, J. E., and Peters, S. P., Insights into IgE-mediated lung inflammation derived from a study employing a 5-lipoxygenase inhibitor. *Prostaglandins*, 50, 1-18 (1995).
- Kim, C. J. and Cho, S. K., Pharmacological activity of flavonoids (III) Structure-Activity relationships of flavonoids in immunosuppression. *Arch. Pharm. Res.*, 14, 147-159 (1991).
- Kim, C. J. and Chung, Z. M., Pharmacological activity of flavonoids (I) Relationships of chemical structure of flavonoids and their inhibitory activity of hypersensitivities. *Yakhak Hoeji*, 34, 348-364 (1990).
- Kim, C. J., Kane, G. C., Zangrilli, J. G., Cho, S. K., Koh, Y. Y., and Peters, S. P., Eosinophils recruited to the lung by segmental antigen challenge show a reduced chemotactic response to leukotriene B₄. *Prostaglandins*, 47, 393-403 (1994).
- Ko, W. C., Shih, C. M., Chen, M. C., Lai, Y. H., Chen, J. H., Chen, C. M., and Lin, C. N., Suppressive effects of 3-O-methylquercetin on ovalbumin-induced airway hyperresponsiveness. *Planta Med.*, 70, 1123-1127 (2004).
- Ko, W. C., Chen, M. C., Wang, S. H., Lai, Y. H., Chen, J. H., and Lin, C. N., 3-O-methylquercetin more selectively inhibits phosphodiesterase subtype 3. *Planta Med.*, 69, 310-315 (2003).
- Ko, W. C., Wang, H. L., Lei, C. B., Shih, C. H., Chung, M. I., and Lin, C. N., Mechanisms of relaxant action of 3-O-methylquercetin in isolated guinea pig trachea. *Planta Med.*, 68, 30-35 (2002).
- Krell, R. D., Osborn, R., Vickery, L., Falcone, K., O'Donnell, M., Gleason, J., Kinzig, C., and Bryan, D., Contraction of isolated airway smooth muscle by synthetic leukotrienes C₄ and D₄. *Prostaglandins*, 22, 387-409 (1981).
- Li, W. G., Zhang, X. Y., Wu, Y. J., and Tian, X., Anti-inflammatory effect and mechanism of proanthocyanidins from grape seeds. *Acta Pharmacol. Sin.*, 22, 1117-1120 (2001).
- Matsumoto, T., Ashida, Y., and Tsukuda, R., Pharmacological modulation of immediate and late airway response and leukocyte infiltration in the guinea pig. *J. Pharmacol. Exp. Ther.*, 269, 1236-1244 (1994).
- Middleton, E. Jr., The Flavonoids. *Trends in Pharmacol. sci.*, 5, 335-338 (1984).
- Middleton, E. Jr. and Drzewiecki, G., Effects of flavonoids and transitional metal cations on antigen-induced histamine release from human basophils. *Biochem. Pharmacol.*, 31, 1449-1453

- (1982).
- Miller, A. L., The etiologies, pathophysiology, and alternative/complementary treatment of asthma. *Altern. Med. Rev.*, 6, 20-47 (2001).
- Morales, M. A., Tortoriello, Meckes, M., Paz, D., and Lozoya, X., Calcium-antagonist effect of quercetin and its relation with the spasmolytic properties of *Psidium guajava* L. *Arch. Med. Res.*, 25, 17-21 (1994).
- Nishino, H., Iwashima, A., Fujiki, H., and Suginuma, T., Inhibition by quercetin of the promoting effect of teleocidin on skin papilloma formation in mice initiated with 7,12-dimethylbenz[a]anthracene. *Jpn. J. Cancer Res.*, 75, 113-116 (1984).
- Omisore, N. O., Adewunmi, C. O., Iwalewa, E. O., Ngadjui, B. T., Adenowo, T. K., Abegaz, B. M., Ojewole, J. A., and Watchueng, J., Antitrichomonal and antioxidant activities of *Dorstenia barteri* and *Dorstenia convexa*. *Braz. J. Med. Biol. Res.*, 38, 1087-1094 (2005).
- Pennock, B. E., Pennock, C. P., Rogers, R. M., Cain, W. A., and Wells, J. H., A noninvasive technique for measurement of changes in specific airway resistance. *J. Appl. Physiol.*, 46, 399-406 (1979).
- Pettinari, A., Amici, M., Cuccioloni, M., Angeletti, M., Fioretti, E., and Eleuteri, A. M., Effect of polyphenolic compounds on the proteolytic activities of constitutive and immunoproteasomes. *Antioxid. Redox. Signal.*, 8, 121-129 (2006).
- Pichurko, B. M., Ingram, R. H. Jr., Sperling, R. I., Lafleur, J. E., Corey, E. J., Austen, K. F., and Drazen, J. M., Localization of the site of the bronchoconstrictor effects of leukotriene C₄ compared with that of histamine in asthmatic subjects. *Am. Rev. Respir. Dis.*, 140, 334-339 (1989).
- Radvanyi, F., Jordan, L., Russo-Marie, F., and Bon, C., A sensitive and continuous fluorometric assay for phospholipase A₂ using pyrene-labeled phospholipids in the presence of serum albumin. *Anal. Biochem.*, 177, 103-109 (1989).
- Ramesh, M., Rao, Y. N., Rao, A. V., Prabhakar, M. C., Rao, C. S., Muralidhar, N., and Reddy, B. M., Antinociceptive and anti-inflammatory activity of a flavonoid isolated from *Caralluma attenuata*. *J. Ethnopharmacol.*, 62, 63-66 (1998).
- Sanjar, S., Aoki, S., Kristersson, Smith, D., and Moley, J., Antigen challenge induces pulmonary airway eosinophil accumulation and airway hyperreactivity in sensitized guinea-pigs: the effect of anti-asthma drugs. *Br. J. Pharmacol.*, 99, 679-686 (1990).
- Shibata, S., Inoue, H., Iwata, S., Ma, R. D., Yu, L. J., Ueyama, H., Takayasu, J., Hasegawa, T., Tokuda, H., and Nishino, A., Inhibitory effects of licochalcone A isolated from *Glycyrrhiza inflata* root on inflammatory ear edema and tumour promotion in mice. *Planta Med.*, 57, 221-224 (1991).
- Shore, P. A., Burkhalter, A., and Cohn, V. H. Jr., A method for the fluorometric assay of histamine in tissues. *J. Pharmacol. Exp. Ther.*, 127, 182-186 (1959).
- Skaltsa, H., Bermejo, P., Lazari, D., Silvan, A. M., Skaltsounis, A. L., Sanz, A., and Abad, M. J., Inhibition of prostaglandin E₂ and leukotriene C₄ in mouse peritoneal macrophages and thromboxane B₂ production in human platelets by flavonoids from *Stachys chrysantha* and *Stachys candida*. *Biol. Pharm. Bull.*, 23, 47-53 (2000).
- Smith, S. F., Page, C. P., Barnes, C. J., and Flower, R. J., Glucocorticosteroids in asthma. *Handbook Exp. Pharmacol.*, 98, 227-252 (1991).
- Strath, M., Warren, D. J., and Sanderson, C. J., Detection of eosinophils using an eosinophil peroxidase assay. Its use as an assay for eosinophil differentiation factors. *J. Immunol. Methods*, 83, 209-215 (1985).
- Toward, T. J. and Broadley, K. J., Early and late bronchoconstrictions, airway hyper-reactivity, leucocyte influx and lung histamine and nitric oxide after inhaled antigen: effects of dexamethasone and rolipram. *Clin. Exp. Allergy*, 34, 91-102 (2004).
- Turner, C. R. and Spannhake, E. W., Acute topical steroid administration blocks mast cell increase and the late asthmatic response of the canine peripheral airways. *Am. Rev. Respir. Dis.*, 141, 421-427 (1990).
- Venge, P., Hakansson, L., and Peterson, C., Eosinophil activation in allergic disease. *Int. Arch. Allergy Appl. Immunol.*, 82, 333-337 (1987).
- Villar, A., Gasco, M. A., and Alcaraz, M. J., Anti-inflammatory and anti-ulcer properties of hypolaetin-8-glucoside, a novel plant flavonoid. *J. Pharm. Pharmacol.*, 36, 820-823 (1984).
- Wardlaw, A. J., Dunnette, S., Gleich, G. J., Collites, J. V., and Kay, A. B., Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Relationship to bronchial hyperreactivity. *Am. Rev. Respir. Dis.*, 137, 62-69 (1988).
- Wu, M. J., Wang, L., Ding, H. Y., Weng, C. Y., and Yen, J. H., *Glossogyne tenuifolia* acts to inhibit inflammatory mediator production in a macrophage cell line by downregulating LPS-induced NF-kappa B. *J. Biomed. Sci.*, 11, 186-199 (2004).
- Yeadon, M., Dougan, F. L., Petrovie, A., Beesley, J. E., and Payne, A. N., Effect of BW B70C, a novel inhibitor of arachidonic acid 5-lipoxygenase, on allergen-induced bronchoconstriction and late-phase lung eosinophil accumulation in sensitised guinea-pigs. *Agents Actions*, 38, 8-18 (1993).