



Antagonistic effects of nobiletin, a polymethoxyflavonoid, on eosinophilic airway inflammation of asthmatic rats and relevant mechanisms

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Abstract

Eosinophils are known to be the important effector cells in asthmatic airway inflammation. The purpose of this study was to investigate the effects of nobiletin, a polymethoxyflavonoid, on eosinophilic airway inflammation of asthmatic rats, and explore its possible mechanisms. Animals were actively sensitized by subcutaneous injection of ovalbumin (OVA). The inflammation in lung tissues of asthmatic rats was observed by hematoxylin and eosin (HE) staining. The eosinophils in blood and BALF were separated by Percoll density gradient centrifugation and counted under microscope. The level of Eotaxin was detected by enzyme-linked immunosorbent assay (ELISA). In addition, the apoptosis of eosinophils was labeled by TdT-mediated dUTP nick end labeling (TUNEL) technique, the semi-quantitative detection for Fas mRNA expression of eosinophils was performed by reverse transcription-polymerase chain reaction (RT-PCR). The airway inflammation of asthmatic rats pretreated with nobiletin was obviously alleviated. Nobiletin (1.5 and 5.0 mg/kg given intraperitoneally) significantly reduced OVA-induced increases in eosinophils, remarkably lowered the level of Eotaxin in blood and broncho-alveolar lavage fluid (BALF) of asthmatic rats. On the other hand, semi-quantitative RT-PCR analysis for Fas of eosinophils from OVA aerosol-challenged sensitized rats showed that Fas mRNA expression of eosinophils was obviously enhanced by nobiletin. Meanwhile, the apoptosis index of cultured eosinophils was significantly elevated after treatment with different doses of nobiletin. These results indicated that nobiletin could inhibit the eosinophilic airway inflammation. Lowering the levels of Eotaxin, relieving airway infiltration of eosinophils and promoting apoptosis of eosinophils by enhancing expression of Fas mRNA may be important mechanisms for nobiletin to antagonize eosinophilic airway inflammation of asthmatic rats.

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Introduction

Bronchial asthma is defined as a chronic airway inflammatory disease characterized by recurrent reversible acute bronchoconstriction and airway hyperresponsiveness (Busse and Lemanske, 2001; Maddox and Schwartz, 2002). The pathophysiology of airway inflammation encompasses eosinophilic infiltration, epithelial denudation, mucus hypersecretion, and airway remodeling. It is attributed to sustained activation of many inflammatory cells, including eosinophils, mast cells, lymphocytes, basophils and macrophages, and synthesis of a variety of proinflammatory mediators and cytokines (Amin et al., 2000). Acute bronchoconstriction is

triggered by release of bronchoconstrictors, including histamine, cysteinyl-leukotrienes, and platelet-activating factor from mast cells upon allergen-induced cross-linking of immunoglobulin E. Airway eosinophilia is induced by a combination of cytokines and chemokines such as interleukin (IL)-5 and Eotaxin. Pulmonary eosinophilia together with airway remodeling, altered neural control of airway tone, and airway epithelial desquamation may contribute to airway hyperresponsiveness in asthma (Wills-Karp, 1999; Kumar, 2001).

Flavonoids are low molecular weight compounds rich in seeds, citrus fruits, olive oil, tea and red wine. As we know, the flavonoids have profound effects on the function of immune and inflammatory cells as determined by a large number and variety of in vitro and some in vivo observations. These ubiquitous dietary chemicals may have significant in vivo effects on homeostasis within the immune system and on the behavior of

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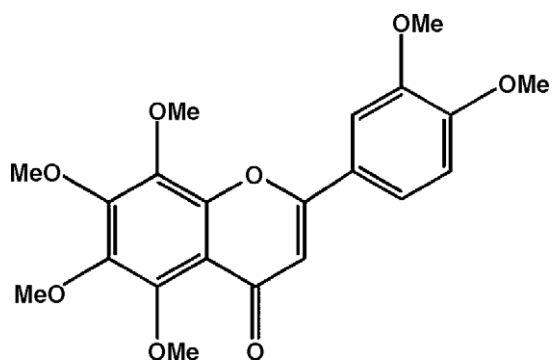


Fig. 1. Chemical structure of nobiletin.

secondary cell systems comprising the inflammatory response. Ample evidence indicates that selected flavonoids, depending on structure, can inhibit secretory processes, mitogenesis, and cell–cell interactions including their possible effects on adhesion molecule expression and function. Moreover, certain flavonoids may affect gene expression and effects of proinflammatory cytokines and cytokine receptors. Some flavonoid effects can certainly be attributed to their recognized antioxidant and radical scavenging properties (Middleton and Kandaswami, 1992). Recent studies demonstrate flavonoids can inhibit degranulation of mast cells, reduce histamine, tryptase, IL-6 and IL-8 release from cultured mast cells, as well as from macrophages (Theoharides et al., 2001; Middleton et al., 2000; Shichijo et al., 2003). In addition, flavonoids are inhibitors of histamine, IL-4 and IL-13 production by activated human basophils (Hirano et al., 2004; Ogasawara et al., 1986). Several flavonoids were found to inhibit the release of histamine, leukotrienes, prostaglandin D₂, and granulocyte macrophage-colony stimulating factor (GM-CSF) from mast cells in a concentration-dependent manner (Chang et al., 2000; Kimata et al., 2000). All the results above suggest that flavonoids may have potent anti-inflammatory and anti-allergic activities especially for mast cell related allergic inflammatory diseases including asthma.

Nobiletin (Shenzhou Jiamei Pharmaceutic Research Corporation, China) is a polymethoxy-flavonoid (Fig. 1) occurring exclusively in citrus fruits, and has been reported to be a promising anti-inflammatory and chemopreventive agent (Chen et al., 1997). We predicted that nobiletin may possess anti-asthmatic airway inflammation effects and may be a new class anti-asthma agent. In this study, we observed the effects of nobiletin on eosinophilic airway inflammation, which plays an important role in the pathogenesis of bronchial asthma. Furthermore, we explored possible mechanisms of nobiletin for anti-airway inflammation induced by eosinophils infiltration.

Methods

Animals

Healthy male Sprague-Dawley (SD) rats 6 to 8 weeks of age and weighing 250 to 300 g were provided by Experimental Animal Center of Nanjing Medical University. Rats were maintained in animal facilities with filtered air. These studies

were carried out in agreement with the official recommendations of The Chinese Community guidelines.

Sensitization and study protocol

Forty animals were randomly divided into five groups (eight in each group): normal control, asthma model and nobiletin (0.5, 1.5, 5.0 mg/kg) groups. Active sensitization was performed by subcutaneous injection of 1 ml saline containing 1 mg of chicken egg OVA (Sigma, USA) and 3.5 mg of aluminum hydroxide gel (PIERCE Co., USA). Rats in normal control were treated with 1 ml of saline only containing 3.5 mg aluminum hydroxide gel instead. In addition, 0.5 ml of *Bordetella pertussis* vaccine containing 2×10^{10} heat-killed organisms was administered intraperitoneally as an adjuvant (Allakhverdi et al., 2000). Two weeks after sensitization, the rats were challenged. Rats in testing groups were injected intraperitoneally with nobiletin 0.5, 1.5, 5.0 mg/kg, respectively daily 1 h before OVA aerosol challenge. Rats in normal control and model group were treated with saline instead. The administration lasts for a week.

Preparations for anticoagulated blood and serum

24 h after the last exposure to OVA aerosol, rats were anesthetized by intraperitoneal injection with sodium pentobarbital (30 mg/kg). The anticoagulated blood was collected using a heparin-coated tube through a cannula inserted into the carotid artery. The other tube untreated with heparin was used to collect blood for serum preparation.

Histopathological observation

The lower lobes of the right lungs were removed, fixed in 10% neutral buffered formalin for at least 24 h, processed routinely. After being embedded in paraffin, the tissues were cut into 5- μ m thick sections and stained with hematoxylin and eosin (HE) before examination under a light microscope (Duan et al., 2003).

Bronchoalveolar lavage

Bronchoalveolar lavage (Duan et al., 2003) was performed 24 h after the last OVA challenge. Rats were anaesthetized by an intraperitoneal injection of pentobarbital sodium (30 mg/kg). Tracheotomy was performed, and a cannula was inserted into the trachea. Phosphate-buffered saline was instilled into the lungs, and BALF was collected. The recovered BALF were pooled and centrifuged, generating a BALF cell pellet and a cell-free supernatant. Cell-free lavage fluid was frozen and stored at -70°C until use.

Eosinophils purification and count

Eosinophils were isolated from the anticoagulated blood and BALF of asthmatic rats after removal of mononuclear cells by Percoll (Pharmacia Co., Sweden) density-gradient centrifugation (Gartner, 1980; Jawien et al., 2004). Then the depletion of neutrophils by immunomagnetic negative selection as previously

described (Masu et al., 2002) was performed. The purity of eosinophils as determined by light microscopic examination of cytopsin preparations stained with Diff-Quik (International Chemical Ltd., Osaka, Japan) was higher than 99%. The viability assessed by trypan blue dye exclusion was higher than 99%. The purified eosinophils were counted under the light microscope.

Eosinophils culture

Freshly purified eosinophils were resuspended at a density of $1 \times 10^6/\text{ml}$ in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) (Cansers International Inc., Toronto, Canada), penicillin G 100 U/ml, and streptomycin (Meiji Seika Co. Ltd, Tokyo, Japan) 100 mg/ml (Way et al., 2005). Eosinophils were cultured at 37 °C in 5% CO₂ in the presence or absence of nobiletin in 96-well flat-bottomed tissue culture plates. After 24 h, the cultured cells were collected. TdT-mediated dUTP nick end labeling (TUNEL) technique was

used to detect apoptosis of eosinophils. 2×10^6 cells were collected to detect the expression of Fas mRNA through reverse transcription-polymerase chain reaction (RT-PCR). The culture was performed in duplicate for each culture condition and all results represent the mean of four experiments.

Measurement of Eotaxin by enzyme-linked immunosorbent assay (ELISA)

The concentrations of Eotaxin in serum and BALF were measured using a ELISA kit (Pharmingen, USA) according to the manufacturer's guideline for users.

TdT-mediated dUTP nick end labeling (TUNEL) of eosinophils

The TUNEL method is based on the specific binding of terminal deoxynucleotide transferase (TdT) to the 3'-OH ends of DNA and the ensuing synthesis of a polydeoxynucleotide

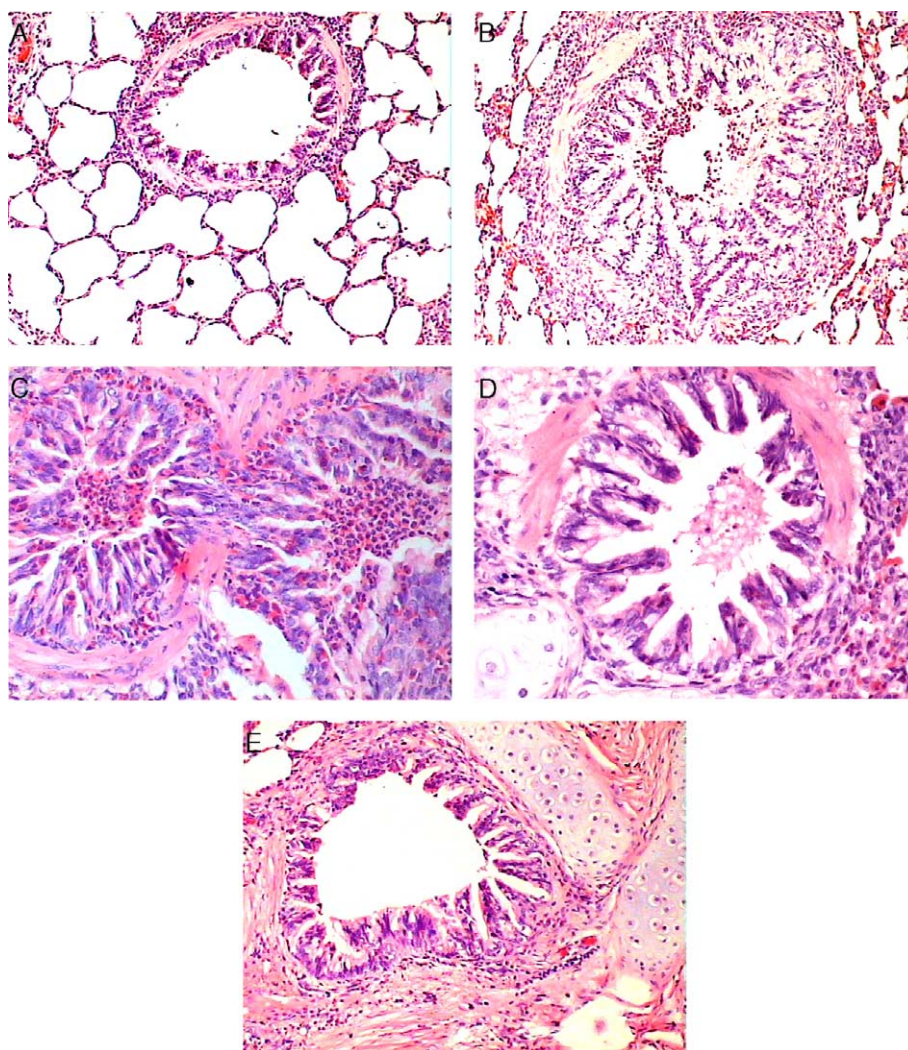


Fig. 2. Histologic examination of inflammatory cell infiltration in the lung tissues from rats after inhalation of saline aerosol (A normal control), OVA aerosol (B, C, D asthma model), OVA aerosol plus 5 mg/kg nobiletin (E nobiletin 5.0 mg/kg). In trachea from rats that had inhaled OVA aerosol, abundant cell infiltration was primarily localized in the lamina propria beneath the pseudostratified respiratory epithelium and mucus hypersecretion was evident. Inflammatory exudates were also observed in the intrapulmonary peribronchiolar tissues. In panel C, OVA-induced eosinophilia in trachea was observed (HE staining. A, B, E magnification $\times 100$; C, D magnification $\times 200$).

Table 1
Effects of nobiletin (0.5, 1.5, 5.0 mg/kg given i.p. 1 h before OVA) on the number of eosinophils in blood and BALF of asthmatic rats induced by OVA challenge

Group	Dose/mg kg ⁻¹	The number of Eos/ $\times 10^6$ /ml	
		Blood	BALF
Normal control	–	1.62 $\pm$ 0.68	1.17 $\pm$ 0.31
Asthma model	–	12.06 $\pm$ 2.35**	11.18 $\pm$ 2.40**
NBL low dose	0.5	10.52 $\pm$ 2.03	9.65 $\pm$ 1.84
NBL medium dose	1.5	8.28 $\pm$ 1.06 [#]	7.25 $\pm$ 0.98 [#]
NBL high dose	5.0	2.88 $\pm$ 0.66 ^{##}	2.35 $\pm$ 0.78 ^{##}

Data are expressed as mean $\pm$ S.D., $n=8$. ** $P<0.01$, vs. normal control; [#] $P<0.05$, ^{##} $P<0.01$, vs. asthma model.

polymer. The TUNEL method was applied to eosinophil smear sections with Apoptosis Detection Kit (Sino–American Biotechnology Company). Eosinophil smear sections were prepared, fixed in 10% neutral buffered Formalin for 10 min at room temperature, rinsed in PBS, and then incubated in 2% H₂O₂ for 15 min to quench endogenous peroxidases. To quantitate the incidence of apoptosis, TUNEL-positive cells were counted. The data were represented as the percentage of TUNEL-positive cells to the total number of eosinophils.

Semi-quantitative RT-PCR for Fas mRNA of eosinophils

Total RNA was extracted from 2×10^6 eosinophils with Trizol reagents (Promega, USA) according to the manufac-

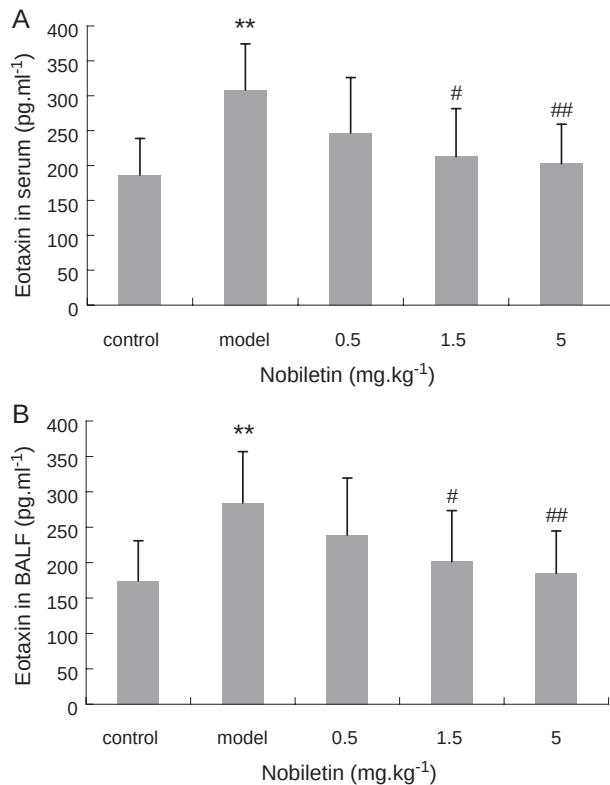


Fig. 3. Effects of nobiletin (0.5, 1.5, 5.0 mg/kg given i.p. 1 h before OVA) on the level of Eotaxin in serum (A) and BALF (B) induced by OVA challenge in sensitized rats. Data are expressed as mean $\pm$ S.D., $n=8$. ** $P<0.01$ vs. normal control, [#] $P<0.05$, ^{##} $P<0.01$ vs. model.

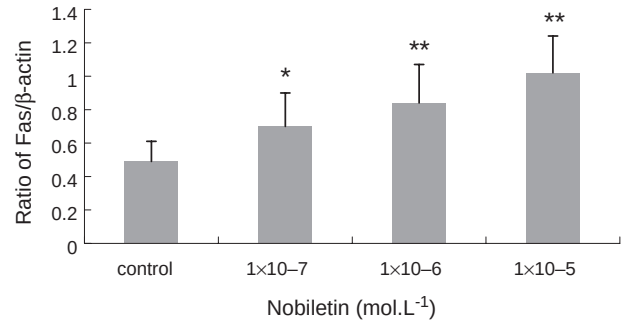


Fig. 4. Effects of nobiletin (1×10^{-7} , 1×10^{-6} , 1×10^{-5} mol/L) on Fas mRNA expression of eosinophils from sensitized rats cultured for 24 h. Ratio of Fas/β-actin denotes expression of Fas mRNA by semi-quantitative measure. Data are expressed as mean $\pm$ S.D., $n=8$. * $P<0.05$, ** $P<0.01$ vs. control.

turer's guidelines. Chemicals for RT-PCR (Bio Basic Inc, Canada) were used as follows. For cDNA synthesis, 20 μ l reverse transcription mixture containing total RNA 1 μ g, dNTP 1 mmol/L, random primer 0.2 μ g, RNasin 20 U, M-MuLV reverse transcriptase 200 U was well mixed and incubated at 42 °C for 60 min, and then the reverse transcriptase was inactivated by heating the mixture at 70 °C for 10 min. Specific primers for rat Fas and β-actin (an internal standard) were synthesized according to published sequences (Roughton et al., 1999; Wang et al., 2004). Fas primers, upstream sequence: 5'CTG CAG ATA TGC TGT GGA TCA 3', downstream sequence: 5'TTT GGT GTT GCT GGT TGG T 3'; β-actin primers, upstream sequence: 5'CCA ACC GTG AAA AGA TGA CC-3', downstream sequence: 5'CAG GAG GAG CAA TGA TCT TG-3'. All primers were synthesized by Takara Co. with the product size of 491bp and 660bp, respectively. PCR reaction constituents were as follows: 2 μ l of cDNA mixture was subjected to amplification in 50 μ l of final volume with MgCl₂ 1.5 mmol/L, dNTPs 0.2 mmol/L, 20 pmol of each primer, and 2 U of Taq DNA polymerase in the reaction buffer.

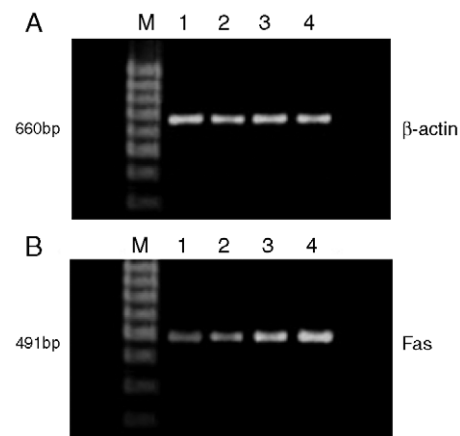


Fig. 5. Effects of nobiletin on Fas mRNA expression of eosinophils from sensitized rats cultured for 24 h. The expression of Fas (B) and β-actin (A) was determined by semi-quantitative RT-PCR. β-actin was used as an internal standard. PCR products of 10 μ l were separated by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining. The density of each band was measured by UVP gel analysis system. M represents marker; 1 represents control; 2 represents 1×10^{-7} mol/L nobiletin, 3 represents 1×10^{-6} mol/L nobiletin, 4 represents 1×10^{-5} mol/L nobiletin.

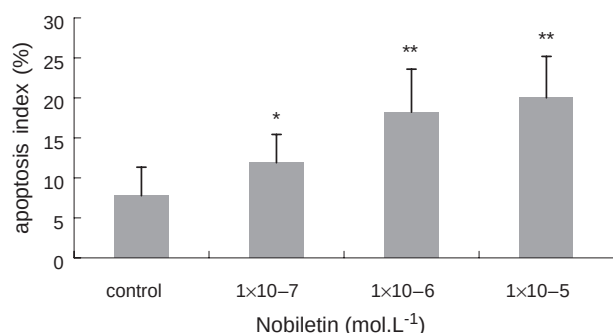


Fig. 6. Effects of nobiletin (1×10^{-7} , 1×10^{-6} , 1×10^{-5} mol/L) on apoptosis of eosinophils from sensitized rats cultured for 24 h. Apoptosis index (TUNEL-positive cells/total cells $\times 100\%$) was presented. Data are expressed as mean $\pm$ S.D., $n=8$, * $P<0.05$, ** $P<0.01$ vs. control.

PCR reaction conditions were as follows: 94 °C, 5 min; then 94 °C, 1 min; 65 °C for Fas, 67 °C for β -actin, 1 min; 72 °C, 45 s, for 35 cycles; and 72 °C 10 min to end the reaction. PCR products of 10 μ l were separated by 1.5% agarose gel electrophoresis and visualized using ethidium bromide staining. The density of each band was measured by UVP gel analysis system. This semi-quantitative measure for Fas was expressed as ratios to β -actin.

Statistics analysis

All values were expressed as mean $\pm$ S.D. The significance for the difference between the groups was analyzed with SPSS 10.0 by one-way ANOVA. Differences were considered significant when $P<0.05$. The correlation was analyzed by SPSS Pearson analysis method.

Results

Effects of nobiletin on histopathological changes in lung tissues

As demonstrated in Fig. 2A, rats in normal control showed normal bronchial and alveolar architecture. Rats in model group exhibited marked lung histopathologic abnormalities, characterized by bronchostenosis, epithelium hyperplasia, leakage of microvessel, bronchial wall edema, mucous plugging and infiltration of inflammatory cells including a number of eosinophils in bronchial and alveolar regions (Fig. 2B, C, D). After treatment with nobiletin at different doses, lung tissues appeared relatively better, with a prominent decrease in inflammation response compared to model group (Fig. 2E).

Effects of nobiletin on the number of eosinophils in asthmatic rats

The results showed that the number of eosinophils in blood and BALF of asthma model rats increased apparently. There was significant difference between model group and normal control ($n=8$, $P<0.01$). Compared to model group, nobiletin at 1.5 and 5.0 mg/kg can decrease the number of eosinophils in asthmatic rats significantly ($n=8$, $P<0.05$ and $P<0.01$, respectively; Table 1).

Effects of nobiletin on Eotaxin level in serum and BALF of asthmatic rats

Compared to normal control, the level of Eotaxin in blood and BALF of asthma model rats increased prominently

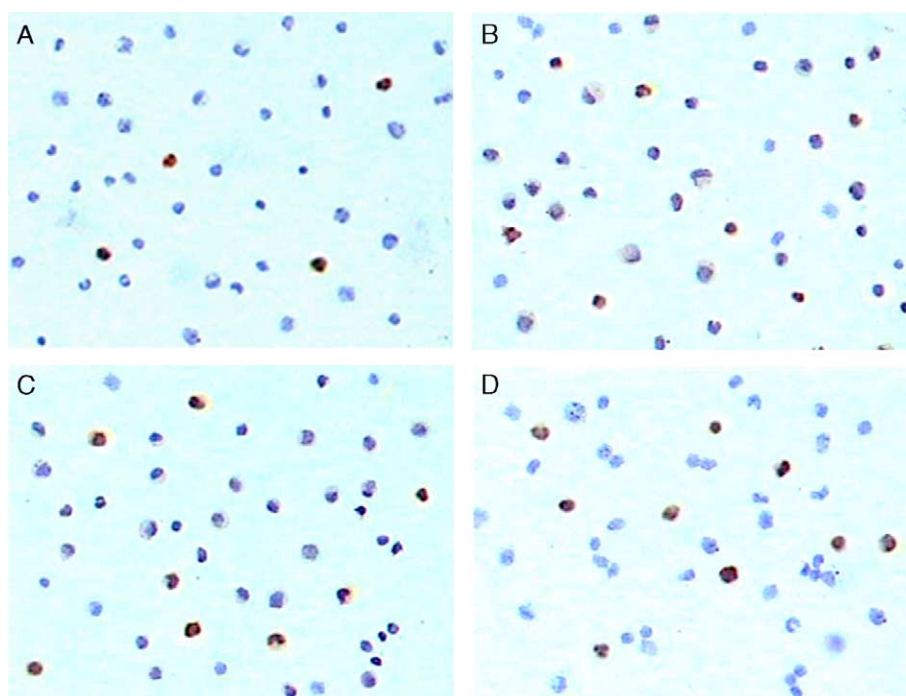


Fig. 7. Apoptosis of eosinophils cultured for 24 h by TUNEL (A, B, C, D magnification $\times 400$) in the absence (A) and presence of nobiletin (B 1×10^{-7} , C 1×10^{-6} , D 1×10^{-5} mol/L). In figure A, a few apoptotic cells were observed. The apoptotic cells increased obviously after treatment with different concentrations of nobiletin (B, C, D).

(308.34 ± 66.0 vs. 186.02 ± 52.75 ; 284.28 ± 72.40 vs. 173.98 ± 56.93 pg/ml; $n=8$, $P<0.01$). Except for low dose of nobiletin, there was significant decrease in level of Eotaxin in serum (212.51 ± 69.06 vs. 308.34 ± 66.0 , $P<0.05$; 202.49 ± 56.75 vs. 308.34 ± 66.0 , $P<0.01$, $n=8$) and BALF (201.16 ± 72.3 vs. 284.28 ± 72.40 , $P<0.05$; 184.85 ± 59.74 vs. 284.28 ± 72.40 , $P<0.01$, $n=8$) after treatment with nobiletin at higher doses compared to model group (Fig. 3).

Effects of nobiletin on Fas mRNA expression in eosinophils

In this study, the expression of β -actin mRNA was detected as an internal standard. The values of this semi-quantitative measure were expressed as ratios of Fas to β -actin. Fas mRNA of eosinophils were expressed in the presence or absence of nobiletin (Figs. 4 and 5). Semi-quantitative RT-PCR data indicated that Fas mRNA expression of eosinophils from sensitized rats was slight. After treatment with different concentrations of nobiletin, a significant increase in expression of Fas mRNA was observed.

Effects of nobiletin on apoptosis index of eosinophils

Eosinophil apoptosis was examined by TUNEL technique. The apoptosis incidence was evaluated by apoptosis index (TUNEL-positive cells/total cells 100%). A small number of cells labeled by the TUNEL method were detected in the control group ($7.79 \pm 3.55\%$). The incidence of eosinophil apoptosis increased 24 h after low concentration of nobiletin exposure ($11.91 \pm 3.52\%$, $n=8$, $P<0.05$ vs. control). A significant increase in the incidence of TUNEL-positive cells was observed in sections from eosinophils 24 h after higher concentrations of nobiletin exposure ($18.21 \pm 5.38\%$; $20.01 \pm 5.18\%$, $n=8$, $P<0.01$ vs. control; Figs. 6 and 7).

Correlation between expression of Fas mRNA and apoptosis of eosinophils

As described above, nobiletin enhanced both the expression of Fas mRNA and the apoptosis index of eosinophils in a concentration-dependent way. By Pearson correlative analysis a significantly positive correlation between the expression of Fas mRNA and the apoptosis index of eosinophils was found ($r=0.824$, $P<0.01$).

Discussion

Allergic asthma is the most common cause of significant respiratory morbidity in the world. It is a chronic disorder characterized by chronic airway inflammation, airway hyper-responsiveness, reversible airway obstruction, airway remodeling and mucus hypersecretion. It has been widely recognized that the infiltration of eosinophils is a hallmark of this disease (Cain, 2001). Eosinophils at the site of inflammation release preformed and newly synthesized mediators, including eosinophil cationic protein, major basic protein, leukotriene C₄, and reactive oxygen. These mediators contribute to the formation of

the pathological features of asthmatic airways such as bronchoconstriction, mucus hypersecretion, microvascular leakage, submucosal edema, and epithelial shedding, which lead to the clinical features of asthma: hyperresponsiveness and airway narrowing. Attenuating accumulation and activation of eosinophils in airway contributes to the relief of airway inflammation (Bousquet et al., 2000).

It has been demonstrated that many flavonoids exert potent antiallergic and anti-inflammatory effects. Previous studies have shown that the flavonoids alleviated bronchoconstriction and airway hyperreactivity in ovalbumin sensitized mice (Das et al., 2003). Flavonoids were also reported to influence monocytic guanosine triphosphatase activity and play protective roles in experimental allergic encephalitis (Hendriks et al., 2004). Moreover, they could inhibit the development of dermatitis and IgE elevation in the allergic mouse model (Kotani et al., 2000). In addition, they could repress TH2-type cytokines production by activated human basophils (Hirano et al., 2004; Higa et al., 2003). In the present study, we firstly report that nobiletin, a polymethoxyflavonoid occurring exclusively in citrus fruits, possesses obvious anti-eosinophilic airway inflammation effects in asthmatic rats. Pretreatment with nobiletin before challenge significantly decreased the number of eosinophils in blood and BALF of asthmatic rats. Meanwhile, the severity of inflammation in asthmatic lung tissues was markedly relieved. Furthermore, we investigated the mechanisms of nobiletin for anti-eosinophilic airway inflammation effects in asthmatic rats.

Eotaxins are a family of CC chemokines that can recruit the inflammatory cells bearing the CCR3 receptor to the sites of allergic inflammation (Rankin et al., 2000). CCR3 is highly expressed on eosinophils (Daugherty et al., 1996; Ponath et al., 1996). The expression of Eotaxin mRNA and protein was found to be increased in the bronchial epithelium and submucosal layer of the airways of chronic asthma (Ying, 2000). Furthermore, the elevation of Eotaxin levels was proportional to eosinophil infiltration and bronchial hyper-reactivity (Ying et al., 1997; Lamkhieoued et al., 1997). Eotaxin is an important factor for eosinophil recruitment to the asthmatic airway which leads to eosinophilic airway inflammation. Our result that nobiletin could significantly decrease the level of Eotaxin in serum and BALF of asthmatic rats highly implies that nobiletin may exert anti-inflammation effect by lowering the level of Eotaxin.

Apoptosis of eosinophils in asthmatic airway is delayed, which aggravates the infiltration of eosinophils in inflammatory airway. Apoptosis is the course of physiologic cell death characterized by morphologic changes such as membrane blebbing and nuclear condensation, and by the degradation of DNA into oligonucleosomesized fragments showing a “ladder” pattern on agarose gel (Tai et al., 1991; Yamaguchi et al., 1991; Stern et al., 1992). Apoptotic eosinophils have been shown to be removed from the tissue without releasing inflammatory mediators after ingestion by macrophages, which is believed to be one of the important mechanisms in the resolution of inflammation (Haslett, 1992). Therefore, the induction of apoptosis in eosinophils would be beneficial in the

treatment of asthma. Our result showed that nobiletin enhances the apoptosis index of eosinophils from asthmatic rats, which may be another pathway for nobiletin to exert anti-eosinophilic airway inflammation effect in asthmatic rats.

Fas ligand/Fas receptor molecular interactions have been implicated as having an important function for the regulation of eosinophil apoptosis (Simon et al., 1998). Fas (CD95, APO-1) is a cell-surface receptor belonging to the nerve growth factor/tumor necrosis factor (TNF) receptor family, whereas its ligand, FasL, belongs to the TNF family (Nagata and Golstein, 1995). FasL is expressed in activated T-cells and macrophages, whereas Fas may be expressed in several target cells. Interaction between FasL and Fas leads to apoptotic death of Fas-expressing cells (Nagata, 1997). Fas was found to be expressed in eosinophils. It was thought that Fas actively participated in the apoptosis of eosinophils, which contributed to the relief of eosinophilic airway inflammation of asthma. In the present study, nobiletin was observed to enhance the expression of Fas mRNA on eosinophils from asthmatic rats. Moreover, the result showed that the increased expression of Fas mRNA was accompanied by the increasing of the apoptosis index of eosinophils. There was a significantly positive correlation between them, which highly implies that nobiletin may promote eosinophil apoptosis by enhancing Fas expression.

In conclusion, our study firstly demonstrated that nobiletin exerted beneficial effects on asthmatic eosinophilic airway inflammation. Lowering the level of Eotaxin and increasing the apoptosis index of eosinophils by enhancing the expression of Fas may be important mechanisms for nobiletin exerting anti-eosinophilic airway inflammation effect in asthmatic rats. Glucocorticoid and selective β_2 receptor agonist are still the predominant drugs in therapy for asthma at present. However, the inevitable side effects of these drugs limited their long-term and large-dose use. Our findings that nobiletin, a polymethoxy-flavonoid occurring exclusively in citrus fruits, reduced eosinophilic airway inflammation raise the possibility that nobiletin may be of high therapeutic value in asthma. It may produce a synergistic effect on asthma therapy with glucocorticoid or selective β_2 receptor agonist. Therefore, it is reasonable to expect that nobiletin might be a new promising therapeutic agent for asthma and other allergic inflammatory diseases.

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