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Naringin attenuates enhanced cough, airway hyperresponsiveness and airway inflammation in a guinea pig model of chronic bronchitis induced by cigarette smoke

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ABSTRACT

Naringin is a flavanone with various bioactivities including expectorant effect, antitussive effect and inhibitory effects on asthma and acute lung injury. In present study we examined the effects of naringin on enhanced cough, airway hyperresponsiveness (AHR) and airway inflammation in chronic cigarette smoke (CS) exposure-induced chronic bronchitis in guinea pigs. To achieve this, guinea pigs were exposed to CS for 8 weeks (10 cigarettes/day, 6 days/week). Oral administration of naringin (9.2, 18.4 and 36.8 mg/kg) significantly attenuated the enhanced cough and AHR in smoke-exposed guinea pigs, reduced the concentrations of interleukin-8 (IL-8), leukotriene B4 (LTB4) and tumor necrosis factor- α (TNF- α) in bronchoalveolar lavage fluid (BALF) and decreased the myeloperoxidase (MPO) activity in both BALF and lung tissue, but did not significantly decrease the leukocytes in BALF. Naringin also improved superoxidase dismutase (SOD) activity in lung tissue and increased the content of lipoxin A4 (LXA4) in BALF in this guinea pig model of chronic bronchitis. These results suggested that naringin exhibited antitussive, anti-AHR and anti-inflammation effects on chronic CS exposure-induced chronic bronchitis in guinea pigs, and may possess novel therapeutic potential in the treatment of chronic bronchitis.

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1. Introduction

Chronic bronchitis is a common disease characterized by persistent cough, phlegm and chronic airway inflammation. Together with emphysema, chronic bronchitis is a major component of chronic obstructive pulmonary disease (COPD) a major growing cause of morbidity [1]. The infiltration of neutrophils along with macrophages and CD8+ T lymphocytes, and the increase of various inflammatory cytokines, chemokines such as interleukin-8 (IL-8), leukotriene B4 (LTB4), tumor necrosis factor- α (TNF- α) in airway contribute to the development of chronic bronchitis [2,3]. The decrease of inflammation resolution capacity in airway may also contribute to the perpetuation of chronic bronchitis. The production of lipoxin A4 (LXA4), a pro-resolving lipid mediator, and the expression of its specific receptor ALX have been found decreased in the airway of moderate and severe COPD and asthma patients [4,5].

Cigarette smoking is considered to be the most important cause of COPD [1]. Poor lung function and chronic cough are strongly

correlated with cigarette smoking [6,7]. The toxic irritant and oxidative material in cigarette smoke (CS) can induce the oxidant–antioxidant imbalance [8] and protease–antiprotease imbalance [9] in pulmonary. Smoke-exposed guinea pig models have been well established and are considered to be useful animal models for COPD as considerable morphologic and physiologic alterations can be induced in guinea pigs by roughly the same dose of smoke exposure as human [10,11]. In addition, guinea pig model is by now the most useful small animal model for cough research [12], which makes it convenient for the study of the enhanced cough in chronic bronchitis.

Naringin, a flavanone derived from grape and citrus fruits has been shown to have respiratory effect recently. Naringin can exert expectorant effect via promoting tracheal cilia movement, inhibiting the over expression and secretion of MUC5AC and MUC5B mucin, and preventing the hyperplasia of airway goblet cells [13]. Naringin also exhibits peripheral antitussive effect in experimental cough models in guinea pigs [14]. Besides, it has been reported that naringin could suppress the AHR and eosinophilic airway inflammation in OVA-induced asthma and attenuate acute neutrophilic airway inflammation in LPS-induced acute lung injury in mice through blocking the NF- κ B pathway [15,16]. However, it's still unknown that whether these respiratory effects of naringin could work on chronic bronchitis, which is a combination of chronic airway neutrophilia, cough, AHR and mucus hypersecretion. Thus we investigated the effects of naringin on chronic CS exposure-induced chronic bronchitis in guinea pigs to assess the potential of naringin in therapeutics of chronic bronchitis.

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2. Methods

2.1. Animal

Male and female Hartley strain guinea pigs (300–350 g) were purchased from Guangdong Medical Laboratory Animal Center (Guangdong, China). The animals were maintained in ordinary animal cages with food and water available *ad libitum*. Animals were randomly divided into 6 groups. All experimental procedures were approved by the Animal Care and Use Committee of the School of Life Sciences, Sun Yat-sen University, PR China. Adequate measures were taken to minimize the pain of experimental animals.

2.2. Chemicals

Capsaicin (30.5 mg, Hubei Artec Carbohydrate Chemistry Co., Ltd., China) was dissolved in 1 ml pure ethanol and 1 ml Tween 80 and then further dissolved in 8 ml physiological saline solution to yield a 0.01 M stock solution [17]. The stock solution was then diluted with saline to produce a 50 μ M working solution. Prednisone acetate tablets were purchased from Guangdong Huanan Pharmaceutical Group Co., Ltd. (Guangdong, China). The normal group and cigarette smoke exposed (CSE) group were treated with vehicle (saline). Naringin was extracted by our laboratory (extracted from *Citrus grandis* 'Tomentosa' by water, deposited in ethanol, with concentrated filtrate obtained after one to ten times of recrystallization, purity > 98.3%, determined by peak area normalization). Prednisone and naringin were dissolved in saline and orally administered at a gavage volume of 5 ml/kg body weight.

2.3. CS exposure-induced chronic bronchitis model

CS exposures were performed using commercially available filter cigarettes (Cocopal brand cigarettes; China Tobacco Guangdong Industrial Co., Ltd., Guangdong, China). According to the product specifications, each cigarette contained 1.2 mg of nicotine and 13 mg of tar. Animals were placed into a stainless steel smoke chamber (80 cm $\times$ 80 cm $\times$ 100 cm) and exposed to CS in a conscious and unrestrained state. They were repeatedly exposed to the smoke of 10 cigarettes/day, 1 h/day, 6 consecutive days/week, for 8 weeks. During the period of CS exposure, the temperature in the chamber was maintained at about 26 °C. Vehicle, prednisone (2 mg/kg, q.d) and naringin (9.2, 18.4, 36.8 mg/kg, q.d.) were orally administered respectively to CSE group, prednisone group and naringin groups 1 h before each CS exposure session. Age-matched, non-smoke-exposed animals were used as normal group animals with 8 weeks of administration of vehicle.

2.4. Measurement of cough and enhanced pause (Penh)

The measurement was performed 24 h after the last CS exposure. All drugs and vehicle were orally administered 1 h before cough test. Conscious animals were individually placed in an unrestrained whole body plethysmograph manufactured for guinea pigs (Buxco, NY, USA). The plethysmograph was connected to a bias flow regulator which withdrew air at a rate of 2.5 L/min. After 2 min of adjustment to the circumstance, animals were challenged with nebulized (Aerogen (Ireland) Ltd, Galway Ireland) aqueous solution of capsaicin (50 μ M) for 2 min at a nebulization rate of 0.5 ml/min. Coughs were recorded by both Biosystem XA software (Buxco Electronics, Sharon, CT) and manually by a blinded observer during the 2 min of capsaicin exposure and for further 8 min after exposure. Penh was also recorded by the Buxco system from the beginning of adjustment period to the end of cough recording period. Penh is an arbitrary dimensionless value that has been shown to correlate closely with airway resistance. The area under the time–Penh curve (Penh–AUC)

during the 10 min of cough recording period was calculated as an indicator of airway responsiveness [18,19].

2.5. Bronchoalveolar lavage

Guinea pigs were sacrificed by bloodletting under pentobarbital sodium (30 mg/kg) anesthesia 4 h after the capsaicin challenge. The trachea was cannulated after the left main bronchus near the bifurcation was ligated. The right lung was lavaged with 5 ml of ice-cold saline for three times via the cannula. The total and differential leukocyte counts of bronchoalveolar lavage fluid (BALF) were carried out by a flow cytometric hematology system (XT-2000iV, Sysmex Corporation, Kobe, Japan) [20,21]. The rest of BALF was centrifuged at 1500 rpm for 10 min at 4 °C and the supernatant was stored at –80 °C until used.

2.6. Histological evaluation

A segment of the non-lavaged left lung was removed and fixed in 10%, neutral-buffered formalin, embedded in paraffin, cut into 2 μ m sections. Representative samples (n = 3) were stained with hematoxylin and eosin (HE). Three different fields for each one tissue sample were checked under light microscopy at a magnification of $\times 10$ by a blinded observer, then the lung inflammation of this animal was semi-scored by the observer. Lung inflammation was graded from 0 (normal) to 3 (severe) in three categories: interstitial inflammation, inflammatory cell infiltration in bronchus, and vascular inflammatory cell infiltration. The total lung inflammation score was calculated by adding up the individual.

The trachea tissue about 1 cm above the tracheal bifurcation was removed and fixed in 10%, neutral-buffered formalin, embedded in paraffin, cut into 2 μ m sections, and representative samples (n = 3) were stained with periodic acid-schiff (PAS). Three different fields for each one tissue sample were checked under light microscopy at a magnification of $\times 40$ by a blinded observer, then the trachea injury of this animal was semi-scored by the observer. Trachea injury was graded from 0 (normal) to 3 (severe) in three categories: mucus secretion, epithelium injury and inflammatory cell infiltration. The total trachea injury score was calculated by adding up the individual.

2.7. Determination of cytokines and chemokines

The contents of IL-8, TNF- α , LTB4 and LXA4 in the supernatant of BALF were measured with enzyme-linked immunosorbent assay (ELISA) kits (RapidBio, CA, USA) and presented as pg/ μ g protein of BALF. The total protein of BALF supernatant was measured with BCA assay kit (Beyotime, Shanghai, China).

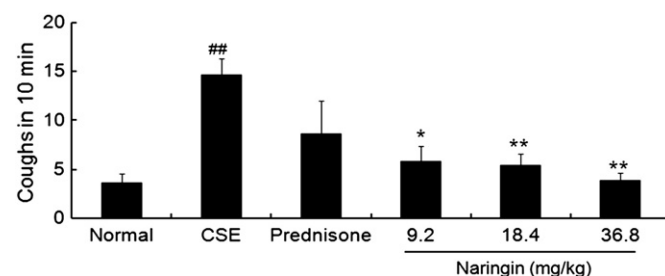


Fig. 1. Antitussive effect of naringin on CS exposure-induced enhanced cough in guinea pigs. Age-matched non-smoke-exposed animals were used as normal group. Prednisone was used at the dose of 2 mg/kg. Each bar represents the mean $\pm$ S.E.M. (** P < 0.01, * P < 0.05, significantly different from normal group; ** P < 0.01, * P < 0.05, significantly different from model group; n = 5–9 animals per treatment group).

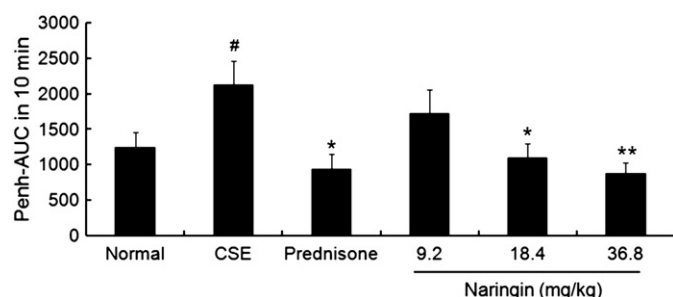


Fig. 2. Effect of naringin on CS exposure-induced AHR. Prednisone was used at the dose of 2 mg/kg. Data represents the area under the time–Penh curve (Penh–AUC) in 10 min. Each bar represents the mean $\pm$ S.E.M.. (# P <0.05, significantly different from normal group; ** P <0.01, * P <0.05, significantly different from model group; n = 5–9 animals per treatment group).

2.8. Assay for superoxide dismutase (SOD) and myeloperoxidase (MPO)

The total SOD activity of lung tissue and the MPO activity of lung tissue and BALF were measured using colorimetric methods with a UV–VIS spectrophotometer (TU-1901, Purkinje General Instrument Co., Ltd., Beijing, China). The assays were conducted using commercial assay kits (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China). The procedures were performed according to the manufacturer's guidance. The total protein of lung tissue was measured with BCA assay kit (Beyotime, Shanghai, China).

2.9. Statistical analysis

Datas are expressed as means $\pm$ S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's t test for multiple comparison. Kruskal–Wallis H test and Mann–Whitney U test were used for the data that failed the Levene's homogeneity of variance test. A P value < 0.05 was taken as significant. Statistical analysis was performed using SPSS for windows 16.0 (SPSS Inc. Chicago, USA).

3. Results

3.1. Antitussive effect of naringin on chronic CS exposure-enhanced cough

Repeated exposure to CS (10 cigarettes/day, 6 days/week) for 8 weeks resulted in a significant increase in capsaicin (50 μ M) aerosol induced coughs in CSE group guinea pigs. Orally administered naringin (9.2–36.8 mg/kg, q.d.) markedly inhibited smoke exposure-induced enhanced cough. Orally administered prednisone (2 mg/kg, q.d.) showed no significant antitussive effect in present study (Fig. 1).

3.2. Effect of naringin on airway responsiveness

Chronic CS exposure led to significant AHR in guinea pigs in present study. Oral administration of naringin (18.4–36.8 mg/kg) significantly suppressed the AHR induced by CS exposure. Treatment with prednisone (2 mg/kg, p.o., q.d.) also significantly inhibited AHR caused by CS exposure (Fig. 2).

3.3. Effect of naringin on CS induced airway leukocyte infiltration

Chronic CS exposure caused significant increase in total leukocyte number in BALF of guinea pigs. As revealed by differential cell count analysis, increased leukocytes in BALF mainly consisted of neutrophils and eosinophils. Oral administration of naringin (9.2–36.8 mg/kg) did not show significant effect on inhibiting the number of total or differential leukocytes in BALF except for the lymphocytes in BALF from animals treated with 18.4 mg/kg of naringin. Oral use of prednisone (2 mg/kg, q.d.) for 8 weeks did not show statistically significant effect on the infiltration of leukocytes in present study either (Table 1).

3.4. Effect of naringin on CS induced lung inflammation and trachea injury

Semi-quantitative scoring of tissue sections of lung and trachea showed that chronic CS exposure caused significant lung inflammation and trachea injury in guinea pigs (Fig. 3c), including mass neutrophil infiltration in alveolus and small bronchial and significant alveolar wall thickening (Fig. 3a). In the trachea sections, obvious hyperplastic and squamous metaplastic and mucus hypersecretion can be found in CSE group animals compared to normal group animals (Fig. 3b).

8-week use of naringin (18.4 and 36.8 mg/kg, p.o., q.d.) significantly inhibited the lung inflammation and trachea injury induced by chronic CS exposure, reducing the accumulation of neutrophils and alveolar wall thickening in lung (Fig. 3a). In addition, epithelial hyperplasia, squamous metaplasia and mucus hypersecretion were relieved in the trachea of naringin-treated animals (Fig. 3b). However, prednisone (2 mg/kg, p.o., q.d.) didn't show significant effect on the lung inflammation and trachea injury in smoke-exposed guinea pigs (Fig. 3a and b). Obvious trachea epithelium exuviation was found in prednisone-treated (2 mg/kg, p.o., q.d.) animals, which may be associated with the apoptosis of epithelial cells induced by prednisone [22].

3.5. Effect of naringin on cytokines and chemokines in BALF

When compared with normal group animals, the contents of inflammatory factors including IL-8, TNF- α and LTB4 in BALF were significantly higher in CSE group animals. However, LXA4, an important pro-resolution mediator, was significantly lower in CSE group animals than that in normal group animals (Fig. 4).

Table 1
Effect of naringin on cigarette smoke induced leukocyte infiltration in BALF.

Group	Total cells (10^4 /ml)	Neutrophils (10^4 /ml)	Lymphocytes (10^4 /ml)	Monocytes (10^4 /ml)	Eosinophils (10^4 /ml)	Basophils (10^4 /ml)
Normal	56.8 $\pm$ 6.1	8.2 $\pm$ 0.5	17.5 $\pm$ 4.8	32.3 $\pm$ 4.1	1.5 $\pm$ 0.7	1.7 $\pm$ 0.5
CSE	81.2 $\pm$ 10.6 [#]	15.8 $\pm$ 0.9 [#]	15.3 $\pm$ 2.6	42.2 $\pm$ 8.2	7.2 $\pm$ 0.7 [#]	0.7 $\pm$ 0.2
Prednisone 2 mg/kg	113 $\pm$ 40	50.5 $\pm$ 27.4	10.8 $\pm$ 2.3	44.5 $\pm$ 12.5	6.3 $\pm$ 1.7	1 $\pm$ 0.4
Naringin 9.2 mg/kg	82.7 $\pm$ 11.6	17.2 $\pm$ 4	12.7 $\pm$ 3.6	38.2 $\pm$ 7.3	13.8 $\pm$ 4.1	0.8 $\pm$ 0.3
Naringin 18.4 mg/kg	67.3 $\pm$ 9.1	12.2 $\pm$ 1.8	8 $\pm$ 1.5 [*]	35.2 $\pm$ 5.3	10.8 $\pm$ 2.7	1.2 $\pm$ 0.2
Naringin 36.8 mg/kg	61.3 $\pm$ 7.2	12 $\pm$ 2.4	10.8 $\pm$ 1.9	35.8 $\pm$ 6.9	5.7 $\pm$ 1.7	1 $\pm$ 0.4

Guinea pigs in CSE group and experimental groups were exposed to cigarette smoke for 8 weeks (10 cigarettes/day, 6 days/week), during which prednisone and naringin were orally administered. Age-matched non-smoke-exposed and vehicle-administered animals were used as normal group animals. Data are expressed as the mean $\pm$ S.E.M. (## P <0.01, * P <0.05, significantly different from normal group; * P <0.05, significantly different from model group; n = 5–9 animals per treatment group).

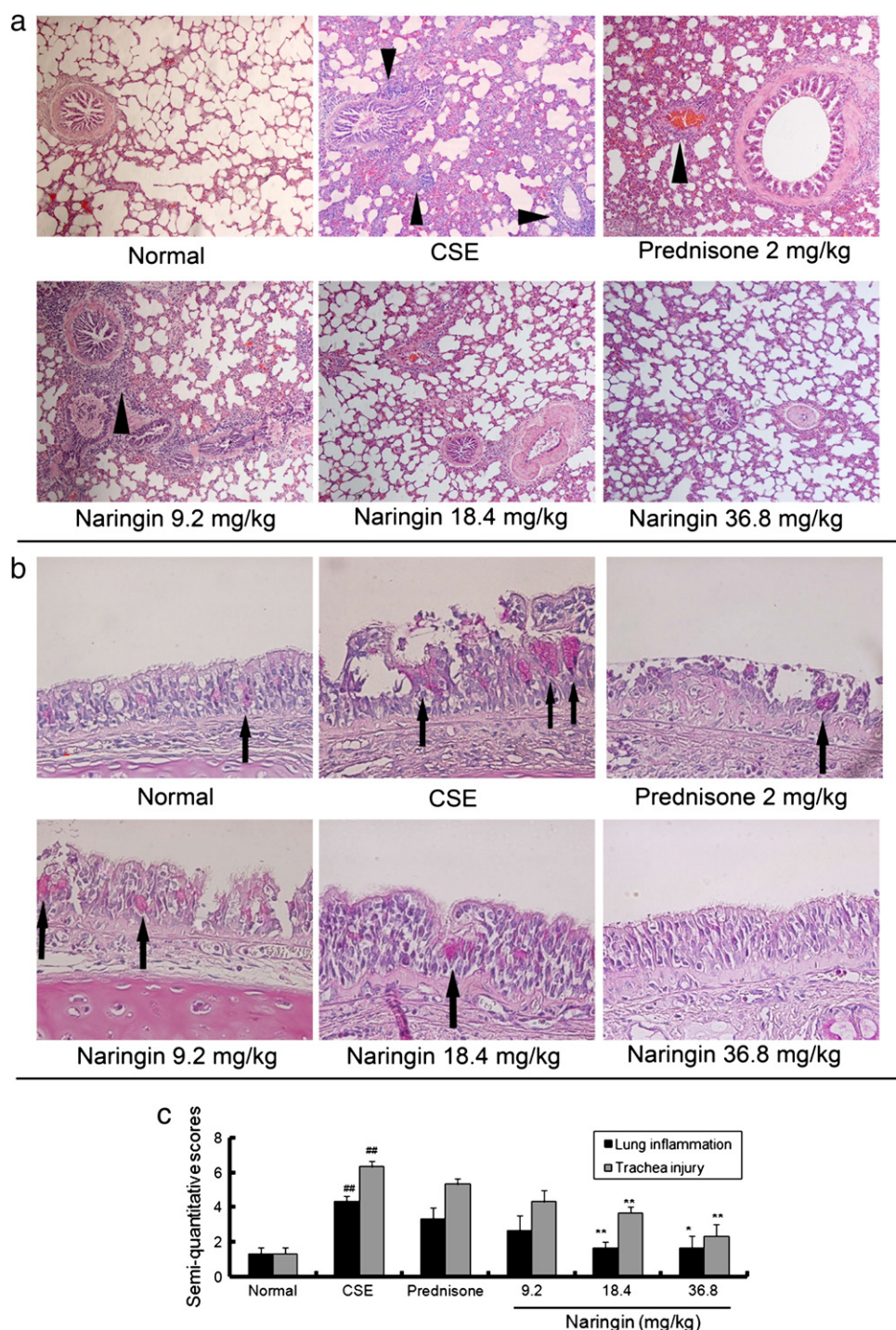


Fig. 3. Effects of naringin on CS induced lung inflammation and trachea injury. Representative micrographs of lung tissue (a, stained with HE, $\times 10$) and trachea (b, stained with PAS, $\times 40$) is shown. The inflammatory cell infiltration is indicated as $\blacktriangle$, and the positive staining of goblet cells is indicated as $\uparrow$. (c) Histological semi-quantitative scores of lung inflammation and trachea injury. Each bar represents the mean $\pm$ S.E.M. ($^{##}P < 0.01$, significantly different from normal group; $^{**}P < 0.01$, $^{*}P < 0.05$, significantly different from model group; $n = 3$ animals per treatment group).

Treatment with naringin (18.4 and 36.8 mg/kg, p.o., q.d.) significantly reduced the contents of IL-8, TNF- α and LTB $_4$ and increased the content of LXA $_4$ in BALF. Prednisone treatment showed no significant effect on these cytokines and chemokines at the dose of 2 mg/kg (p.o., q.d.).

3.6. Effect of naringin on the activity of MPO in lung tissue and BALF

The activity of MPO in lung tissue and BALF was significantly increased in CSE group guinea pigs. It demonstrated that chronic exposure of guinea pigs to CS significantly enhanced neutrophil

activity in airway lumens and aggravated the inflammation in lung tissue. Treatment with 18.4 mg/kg and 36.8 mg/kg of naringin markedly reduced the activity of MPO in BALF and lung tissue. In contrast, oral administration of 2 mg/kg of prednisone showed no significant effect on the activity of MPO in both samples (Fig. 5).

3.7. Effect of naringin on the activity of SOD in lung tissue

In this study, the activity of SOD in lung tissue of CSE group was significantly decreased. Treatment with naringin (9.2–36.8 mg/kg)

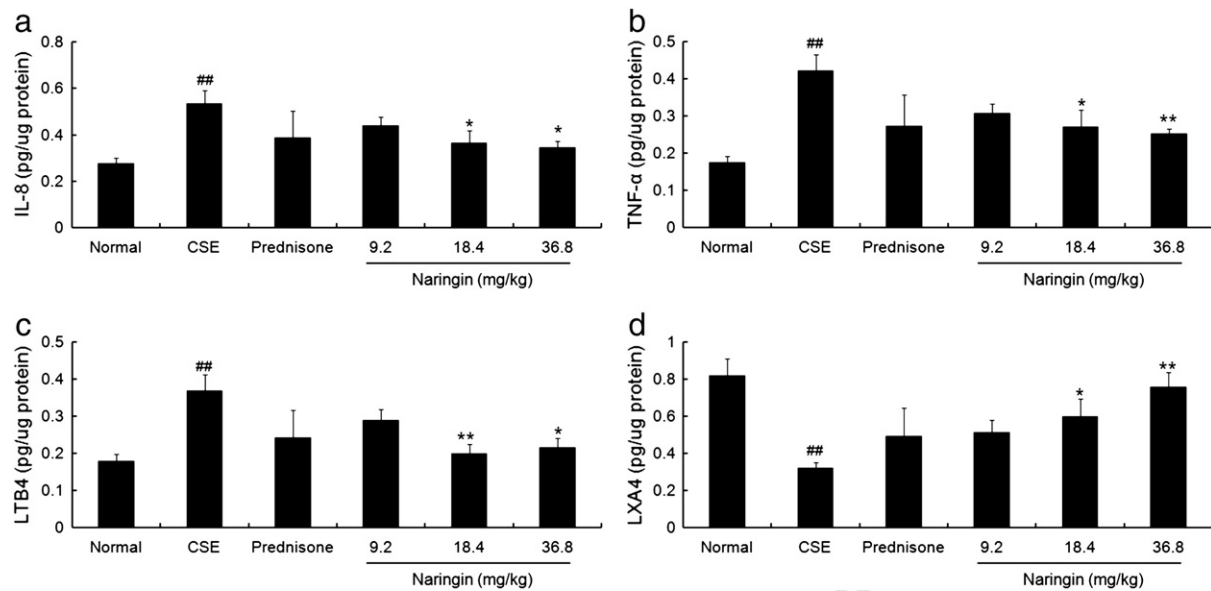


Fig. 4. Effects of naringin on cytokines and chemokines in BALF. Prednisone was used at the dose of 2 mg/kg. Panels a, b and c illustrate the decreasing effect of orally administered naringin on the contents of IL-8, TNF-α and LTB4. Panel d illustrates the increasing effect of orally administered naringin on the contents of LXA4. Each bar represents the mean ± S.E.M. (^{##}*P*<0.01, ^{*}*P*<0.05, significantly different from normal group; ^{**}*P*<0.01, ^{*}*P*<0.05, significantly different from model group; *n*=5–9 animals per treatment group).

markedly increased the activity of SOD in lung tissue compared to CSE group animal. Prednisone showed no effect on the activity of SOD in lung (Fig. 6).

4. Discussion

Here, we investigated the effect of naringin on cough hyperactivity, AHR and airway inflammation of chronic CS-exposed guinea pigs. Repeated exposure of guinea pigs to CS for 8 weeks caused significant cough hyperresponsibility and AHR to capsaicin, induced airway inflammation, epithelium injury, mucus hypersecretion and lung

interstitium hyperplasia, reduced the content of pro-resolution mediator and antioxidant capacity of pulmonary. Given these findings, this guinea pig model is adequate for predicting the efficacy of potential disease-modifying agents for chronic bronchitis.

Via up-regulating the expression of neurokinins, inactivating airway neutral endopeptidase and enhancing the responsiveness of rapidly adapting receptor (RAR) to substance P, both acute and chronic smoke exposure can accelerate the sensitivity of cough reflexes and bronchoconstriction [18,23–25]. Smoke-exposed guinea pigs with enhanced cough are suitable for assessing the antitussive effect of candidates on pathological cough [26]. The peripheral antitussive effect of naringin on non-pathological cough had been confirmed in healthy guinea pig models [14]. Here we further demonstrated the antitussive effect of naringin on enhanced cough and inhibiting effect on AHR in this guinea pig model of smoke-induced chronic bronchitis. However, how the smoke-induced neurogenic inflammation contributes to these, if any, still needs further investigation.

The anti-inflammation effects of naringin in pulmonary diseases have been shown in animal models of asthma [15] and acute lung injury [16], which may be relative with the blockade of NF-κB pathway. However, the effect of naringin on the airway inflammation of chronic bronchitis, a disease mainly consists of chronic airway neutrophilia, has not been reported previously. Interactions between risk factors

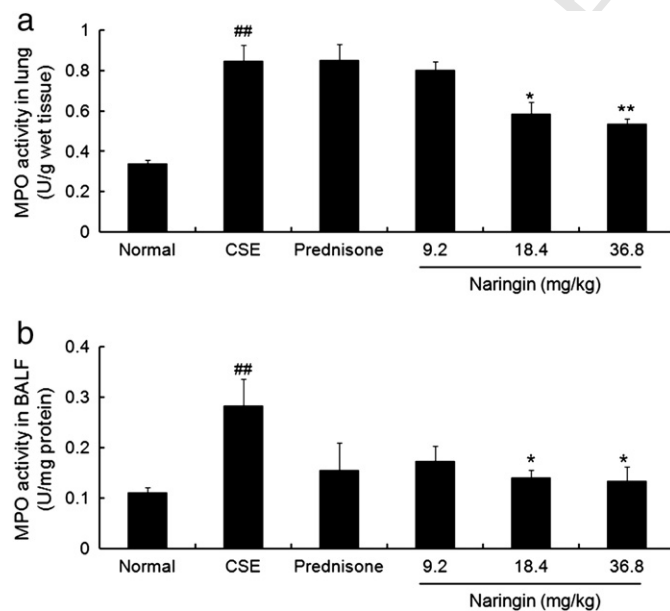


Fig. 5. Effects of naringin on the activity of MPO in lung tissue and BALF. Prednisone was used at the dose of 2 mg/kg. Panel a illustrates the inhibitory effect of orally administered naringin on the activity of MPO in lung tissue. Panel b illustrates the inhibitory effect of orally administered naringin on the activity of MPO in BALF. Each bar represents the mean ± S.E.M. (^{##}*P*<0.01, significantly different from normal group; ^{**}*P*<0.01, ^{*}*P*<0.05, significantly different from model group; *n*=5–9 animals per treatment group).

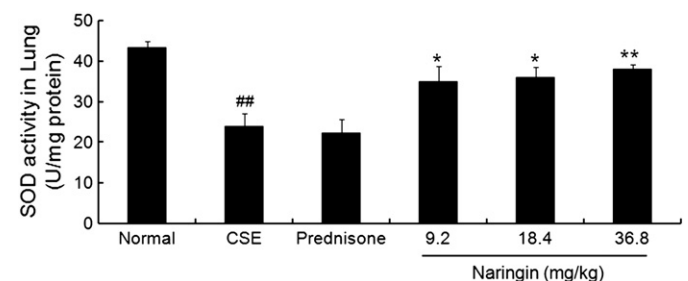


Fig. 6. Effect of naringin on the activity of SOD in lung tissue. Prednisone was used at the dose of 2 mg/kg. Orally administered naringin showed an increasing effect on the activity of SOD in lung tissue. Each bar represents the mean ± S.E.M. (^{##}*P*<0.01, significantly different from normal group; ^{**}*P*<0.01, ^{*}*P*<0.05, significantly different from model group; *n*=5–9 animals per treatment group).

like CS and airway macrophages and epithelium could induce the release of IL-8, LTB4 and TNF- α , which activates the recruitment, infiltration and activation of airway neutrophils. The productions of active airway neutrophils including proteases, oxidants, MPO, IL-8 and LTB4 in turn induce inflammation persistence, mucus hypersecretion and tissue injury in chronic bronchitis [27]. It has been reported that the contents of IL-8, LTB4 and TNF- α in sputum were closely relative with the severity of COPD [28,29]. In present study, 8-week use of naringin did not significantly decrease the number of total leukocytes and neutrophils in BALF of chronic smoke-exposed guinea pigs. However, naringin had markedly reduced the quantities of IL-8, TNF- α , LTB4 and the MPO activity in BALF. The MPO activity in lung tissue, a quantitative index for the number of active neutrophils in lung tissue [30], was also decreased by naringin. These positive results were in accordance with the milder epithelium injury, goblet cell hyperplasia and lung interstitium hyperplasia found in histological examinations. These findings suggested that though not inhibiting the transepithelial egression of leukocytes in airway tissue, naringin had attenuated the inflammation activity of infiltrated cells in both airway lumen and tissue, resulting in less lung tissue inflammation and trachea injury in chronic smoke-exposed guinea pigs.

One noteworthy fact is that 8-week use of prednisone (2 mg/kg, p.o.) did not significantly relieve the CS-induced airway tissue injury (Fig. 3) nor did it show statistically significant anti-inflammation effect in chronic smoke-exposed guinea pigs. Our results complied with the reports about the limited efficacy and high risk of side effects of glucocorticoids in patients with COPD [31,32] and in animal model of COPD [33]. The failure of glucocorticoids in treatment of COPD is thought to be partly due to the decrease of expression of histone deacetylase (HDAC)-2 [34]. The infiltration-enhancing effect and anti-apoptosis effect on neutrophils [35,36] and the apoptosis-inducing effect on epithelial cells [22] may also contribute to the side effects of long-term use of glucocorticoids.

The resolution of inflammation is crucial for maintaining the balance between immune defense effect and tissue injury effect of inflammation. Abnormal resolution of acute inflammation may result in the chronicity of inflammation or even autoimmunity [37]. LXA4, an important mediator for inflammation resolution, has bioactivities on inhibiting recruitment and activation of neutrophil and eosinophil in inflamed site, stimulating non-inflammatory phagocytosis of apoptotic cells, reducing angiogenesis and fibrosis [38]. It has been reported that the production of LXA4 and the expression of its specific receptor ALX decreased in the airway of moderate or severe COPD and asthma patients [4,5]. LXA4 analogue and transgenic expression of human LXA4 receptor could inhibit the airway eosinophilic inflammation and airway hyper-responsiveness in a murine model of asthma [39]. In present study, prophylactic use of naringin remarkably prevented the decrease of LXA4 in BALF of guinea pigs chronically exposed to CS, which is good for the resolution of airway inflammation. The retained LXA4 in naringin treated animals might have contributed to the low inflammation activity in respiratory tracts and the decrease of neutrophil infiltration in lung tissue.

Cigarette smoke can cause extensive ROS formation and up-regulate the expressions of pro-inflammatory mediators through the activation of stress kinases and redox-sensitive transcription factors, such as NF- κ B and activator protein-1 [8]. The decrease of SOD in BALF from smokers has been reported previously [40] and exogenous SOD treatment can inhibit smoke-induced leukocyte adhesion to endothelium [41] and reduce airway neutrophil accumulation in smoke-exposed animal models [42]. The preventive effects of naringin on the decrease of SOD in liver of cholesterol-fed rabbit [43], heart of isoproterenol-induced rat [44] and brain of rat with ischemic reperfusion cerebral injury [45] have been reported. In present study, treatment of naringin significantly increased SOD activity in lung tissue of chronic smoke-exposed guinea pigs, which would be helpful for attenuating the development of chronic bronchitis.

5. Conclusions

In summary, we report here the multi-prong effects of naringin in a guinea pig model of chronic bronchitis induced by chronic CS exposure. Our results showed that naringin effectively reduced the chronic smoke exposure-induced enhanced cough, AHR, airway inflammation activity, and inhibited the decline of SOD activity and LXA4 content of airway in this guinea pig model. These findings showed that naringin might play a valuable role, clinically, in controlling chronic bronchitis.

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