ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY 38 (2014) 279–287

Available online at www.sciencedirect.com

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Mucoactive effects of naringin in lipopolysaccharide-induced acute lung injury mice and beagle dogs

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ARTICLE INFO

Article history:
Received 26 November 2012
Received in revised form
26 April 2014
Accepted 29 April 2014
Available online 9 May 2014

Keywords:
Mucoactive effect
Naringin
LPS
Acute lung injury
Mice
Beagle dogs

ABSTRACT

Our previous study has demonstrated that naringin attenuates EGF-induced MUC5AC hypersecretion in A549 cells by suppressing the cooperative activities of MAPKs/AP-1 and IKKs/IκB/NF-κB signaling pathways. However, the volume of airway mucus is determined by two factors including the number of mucous cells and capacity of mucus secretion. The aim of the present study is to explore the mucoactive effects of naringin in lipopolysaccharide (LPS)-induced acute lung injury (ALI) mice and beagle dogs. The results demonstrated that naringin of 12.4 mg/kg treatment significantly decreased LPS-induced enhancement of sputum volume and pulmonary inflammation, remarkably increased the subglottic sputum volume and solids content in sputum of lower trachea, while partially, but not fully, significantly increased the elasticity and viscosity of sputum in lower trachea of beagle dogs. Moreover, the MUC5AC content in BALF and goblet-cells in large airways of LPS-induced ALI mice were significantly attenuated by dexamethasone (5 mg/kg), ambroxol (25 mg/kg), and naringin (15, 60 mg/kg). However, the goblet-cells hyperplasia in small airways induced by LPS was only significantly inhibited by dexamethasone and naringin (60 mg/kg). In conclusion, naringin exhibits mucoactive effects through multiple targets which including reduction of goblet cells hyperplasia and mucus hypersecretion, as well as promotion of sputum excretion.

Abbreviations: ALI, acute lung injury; BAL, bronchoalveolar lavage; BCA, bicinchoninic acid; LPS, lipopolysaccharide; LT, lower trachea; MTV, tracheal mucociliary velocity; SG, subglottic; RLL, right low lobe; RML, right middle lobe; RHL, right high lobe; LHL, left high lobe.

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http://dx.doi.org/10.1016/j.etap.2014.04.030
1382-6689/

1. Introduction

Naringin (4′,5,7-trihydroxy flavanone-7-rhamnoglucoside) belongs to a family of C6–C3–C6 polyphenol compounds and is abundant in grapefruit and citrus fruits which responsible for the bitter taste of the fruit (Jagetia et al., 2003). Previous studies have demonstrated that naringin has broad spectrum of pharmacological properties which including anti-inflammatory (Liu et al., 2011; Kanno et al., 2006), anti-oxidant (Golechha et al., 2011; Kumar et al., 2010), and mucoregulator effects (Nie et al., 2012). Our previous study has revealed a potential mechanism of naringin against MUC5AC hypersecretion which mediated by suppressing the cooperative activities of MAPKs/AP-1 and IKKs/IκB/NF-κB signaling pathways in EGF-induced A549 cells (Nie et al., 2012). Moreover, naringin was reported to be a potent PDE inhibitor which means being able to increase the content of cAMP and thus activate cAMP-dependent signaling pathways and ion channels related to mucus and serous secretion (Dallas et al., 2008). These results suggested that naringin may be a potent mucoactive compound. However, there are no *in vivo* studies related to the mucoactive effects of naringin. Moreover, the volume of airway mucus is determined by two factors including the number of mucous cells in airway and the mucus secretion capacity of these mucous cells. It remains unclear whether naringin could inhibit airway goblet cells proliferation and hyperplasia in pathological conditions or not.

Acute lung injury (ALI) is a clinical syndrome in which patients develop progressive pulmonary gas exchange defects and severe pulmonary mechanical dysfunction (Maniatis et al., 2008). The pathogenesis of ALI is characterized by disorders of oxidant/anti-oxidant and inflammation/anti-inflammation, increases release of chemokines and cytokines, mucus hypersecretion and plugging, as well as interstitial edema, which were widely reproducible displayed by intratracheal instillation of bacterial LPS (Zhang et al., 2011; Liu et al., 2008). Previous studies have well demonstrated that mucin secretion in tracheal of ferrets was increased by 63% after induced by LPS of 10 mg/kg, while the tracheal mucociliary velocity (MTV) was decreased by 31% (Abanses et al., 2009). Moreover, LPS of 1 μg/mL treatment significantly increased the spinability of mucus in implanted tracheas of rats, which equivalent to the spinability of nasal mucus from patients with chronic sinusitis (Kitano et al., 2011). Besides, the hypersecretion and enhancive spinability of mucus, as well as the decrease of MTV and sputum excretion, would aggravate the possible risk of airways plugging that resulted in impairment of respiratory function and pulmonary gas exchange (Evans and Koo, 2009).

Therefore, the current study was aimed to investigate the mucoactive effects of naringin in LPS-induced ALI mice and beagle dogs, through measuring some indexes such as MUC5AC secretion, airway epithelial goblet cells hyperplasia and mucus secretion, sputum excretion, sputum properties, as well as the wet/dry ratio of lung.

2. Materials and methods

2.1. Materials

LPS (O55:B5, L2880) was purchased from Sigma-Aldrich (St. Louis, Mo, USA). Naringin (Nar) was extracted from *Citrus grandis* 'Tomentosa', purity >99%, determined by peak area normalization. Ambroxol (Amb) was purchased from Shanghai Bolinge Yingehan Pharmaceutical Co., Ltd., batch number 15050301. Dexamethasone (Dex) was purchased from Guangdong Zhongsheng Pharmaceutical Co., Ltd., batch number 20110504. Bicinchoninic acid (BCA) protein assay reagent kit was purchased from Beyotime (Shanghai, China). Commercial MPO and iNOS assay kits were purchased from Jiancheng (Jiancheng, Nanjing, China). Mice MUC5AC ELISA kit was purchased from USCNK (USCNK, Wuhan, China). Canine TNF-α and IL-8 ELISA kits were obtained from R&D Corporation (R&D Systems Inc., Minneapolis, MN, USA). No. 8.0 endotracheal tubes (diameter of 8.0 mm) were purchased from Wellheads (Welllead, Guangzhou, China). The cytology brushes (diameter of 1.8 mm with biopsy channel of 1.0 mm) were purchased from Anrui (Anrui, Hangzhou, China).

2.2. Canine ALI model

2.2.1. Animals and ethics statement

Eighteen beagle dogs (one-year-old, 10 males and 8 females) weighing between 10 and 13 kg were obtained from Guangzhou Institute of Pharmaceutical Industry (Guangzhou, China). The study protocol was approved by the Ethics Committee of Guangzhou Institute of Pharmaceutical Industry (No. 2011061702). The procedures including drugs administration, anesthesia, surgical operation, as well as dispose of the body of dogs were completed by technical personnel of Guangzhou Institute of Pharmaceutical Industry, and all efforts were made to minimize pain of experimental animals.

2.2.2. Canine ALI model preparation

The details of this model have been previously described (Szczepaniak et al., 2008). Briefly, eighteen beagles dogs were randomly divided into three following groups ($n = 6$): (i) Normal control group (Con), (ii) LPS group (LPS), (iii) LPS + Nar group. First, the LPS + Nar group dogs were pre-orally administrated with naringin of 12.4 mg/kg in a capsule, while dogs in Con and LPS groups were pretreated with a placebo capsule. 1 h latter, dogs were intubated with No. 8.0 endotracheal tubes using standard oral intubation techniques after anesthetized with sodium pentobarbital (25 mg/kg bolus) and paralyzed with pancuronium (3 mg bolus). After that, beagles in LPS and LPS + Nar groups were instilled with LPS (4 mg/kg, diluted in 10 mL saline) intrabronchially into each of five lobar bronchi (2 mL aliquots for each), through a flexible 18-G catheter *via* the working channel of a flexible fiberoptic bronchoscope. Beagles in Con group were administrated with equal volume of saline. During the next 8 h of modeling period, the dogs breathed spontaneously and were fluid supported with sodium pentobarbital (60 mg/h) and pancuronium (0.5 mg/h) in 250 mL saline *via* a forelimb *i.v.* catheter. Rectal temperature was