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The expectorant activity of naringenin

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Abstract

The expectorant activity of naringenin was studied. Mucus secretion was evaluated in mice by measuring the tracheal output of phenol red. Mucociliary movement function was investigated using a migration method of carbon granules in unanesthetized pigeons. And the effect of naringenin on the secretion of mucin and lysozyme was performed in the rat tracheal ring explants. Naringenin could significantly increase the secretion of phenol red from mouse tracheas at the doses of 30–67 mg/kg (i.g.) ($P<0.05$). Naringenin, at the dose of 90 mg/kg, increased the tracheal mucociliary velocity (TMV) to 144.4% of control ($P<0.01$). 100 μM naringenin could enhance the basal lysozyme secretion, but had no effect on the basal mucin secretion from the rat tracheal ring explants. Treatment with naringenin at higher concentration (10 μmol/l) could inhibit the 100 ng/ml lipopolysaccharide (LPS)-induced mucin increase. These data suggest, therefore, that naringenin has the expectorant activity.

Keywords: Naringenin; Expectorant; Mucin; Lysozyme; Phenol red; Tracheal mucociliary velocity

1. Introduction

Exocarpium Citri Grandis is a known Traditional Chinese Medicine that used as antitussive and expectorant herbs for thousands of years in China. Naringenin is the main metabolite of naringin which is the main flavonoid in Exocarpium Citri Grandis. Naringenin glucuronide is the main existent form in rat plasma after oral administration of naringin [1]. So, we wondered whether naringenin had expectorant activity or not.

Airway mucus is a non-homogeneous and viscoelastic gel consisting largely of water, a little salt and proteins secreted by serum cells of submucosal glands and smaller quantities of mucin secreted by surface epithelial goblet cells [2]. The mucus that overlies the airway epithelia contributes to protect from inhalation of airborne irritants, particles, and microorganisms by trapping foreign material in its viscous matrix and acting as the medium by which cilia clear agents from the respiratory tract [3]. The imbalance between mucin and volume (water and salt) could lead to reduced mucus clearance in chronic obstructive pulmonary disease and asthma because the efficacy of mucociliary beat could be reduced by increased mucus viscosity. The mucus retention could induce inflammation and cough [4]. To redress the imbalance of mucin and volume to restore the airway surfaces to health, the different approaches of reduction of mucin secretion and addition of volume to thin mucus and promotion of mucociliary clearance can be envision [5].

In this study, we aimed to investigate whether naringenin had effects on the tracheal output of phenol red in mice, and whether naringenin could increase the rate of mucociliary clearance in pigeons, as well as whether naringenin could have effect on mucin and lysozyme secretion from the rat tracheal ring explants *in vitro*.

2. Materials and methods

All protocols were approved by the institutional ethics committee.

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2.1. Materials

Naringenin (purity >95%), penicillin G, streptomycin sulfate and LPS (from Escherichia coli 055:B5), dolichos biflorus agglutinin (DBA), peroxidase conjugated DBA, mucin (from porcine stomach), *Micrococcus lysodeikticus*, adrenaline (Adr) and dexamethasone (Dex) were purchased from Sigma Chemical (St. Louis, MO, USA). The tetramethylbenzidine and lysozyme (from chicken egg white) were purchased from MBChem. Other chemicals and solvents were of analytical-reagent grade and were used without further purification.

2.2. Animals

Male and female Kunming mice weighing 18–22 g were used in the mucus secretion experiment and were purchased from South Medical University. Pigeons of either sex weighing 320–420 g were purchased from the market. Pigeons were checked by the Animal Epidemic Prevention Place of Guangzhou and were free of any infections. Male and female SD rats weighing 280–320 g were used in the tracheal organ culture and were purchased from Guangdong Medical Laboratory Animal Center. All mice and rats were housed at room temperature (22–24 °C) and constant humidity (50–60%) under a 12 h light–dark (07:00–19:00 and 19:00–07:00) cycle.

2.3. The tracheobronchial secretion assay in mice

The procedures were performed as described previously [6,7]. Briefly, oral administration of test compounds, NH_4Cl, ambroxol and vehicle (PEG 400: saline = 1:1) 30 min before intraperitoneal injection of phenol red solution (5% saline solution, 0.1 ml/10 g body weight). Then 30 min after application of phenol red, the mice were anesthetized with pentobarbital at the dose of 75 mg/kg body weight and exsanguinated by cutting the abdominal aorta. After dissected free from adjacent organs, the trachea was removed from the thyroid cartilage to the main stem bronchi and put into 2 ml normal saline immediately. In order to let the naringerin secreting to the tracheal lumen of mice resolve to the medium completely and stably, the tracheas were stored at 4 °C for 12 h. Finally 0.1 ml NaOH (1 mol/l) was added to the saline and optical density of the mixture were measured at 546 nm using TU-1901 dual-beam UV–vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., China).

2.4. Tracheal mucociliary velocity (TMV)

Surgical operation and the following procedures were performed as described previously [8,9]. In brief, a pigeon was fixed in supine position. The trachea was exposed from approximately 0.5 cm below the larynx. Two threads were tightly laid under the exposed trachea, with a distance of 3 cm between the threads, so that the part of the trachea used for the test was kept level. Five microlitre Zhonghua ink (Peking Yidege ink Co., Ltd., Beijing), which was used as the source of carbon particles, was injected into the mucosa layer of trachea with micro-injector (Shanghai Yibo Glass Instuments Ltd. Co., Shanghai). TMV was determined by observing the rate of carbon particle transport in the trachea approximately 75 min after administration of naringenin, NH_4Cl or vehicle (PEG 400: saline = 1:1).

2.5. Preparation of tracheal tissues of rats

The experiment was carried out according to previous report [10] with minor modifications. For removal of tissue, each rat was anesthetized with pentobarbital and exsanguinated by cutting the abdominal aorta. Its trachea was exposed bloodlessly, dissected free from adjacent organs, and removed from the thyroid cartilage to the main stem bronchi. The trachea was then cut into about 1–2 mm length section for making a ring, yielding explants weighing about 3 mg. Every two pieces were in a well and the pieces in 24-well culture dish were grown in DMEM nutrient mixture (GIBCO) supplemented with 10% calf bovine serum (PAA, Switzerland), 100 units/ml penicillin G, and 100 μg/ml streptomycin sulfate. The explants were cultured in a humidified incubator at 37 °C under 5% CO_2 and 95% O_2. Cultures were transferred to fresh medium without serum after 24 h.

2.6. Stimulation of mucin and lysozyme release from the tracheal tissues of rats

After culture in the media with 10% calf bovine serum for 24 h, tracheal pieces were first washed twice with PBS and then incubated with fresh medium without serum for 2 h. For basal secretion experiment, the pieces were incubated with naringenin, Adr, Dex and vehicle (3‰ alcohol) in fresh medium without serum for 24 h before supernatant of each well being collected for determination of mucin and lysozyme release. For LPS challenge experiment, the pieces were incubated at 37 °C for 24 h in fresh medium without serum containing various concentrations of naringenin, Adr, Dex and vehicle (3‰ alcohol), with LPS pretreated at 37 °C for 30 min. All supernatants collected were stored in Eppendoffs at −20 °C until use.

2.7. Development of enzyme linked lectin assay (ELLA)

An ELLA procedure was developed for measuring mucin in culture supernatants [11,12]. A 96-well plate was coated with 60 μl DBA (6 mg/l) overnight at 4 °C. After washing with high salt PBS containing 0.5 mol/l NaCl and 0.1% Tween-20, 50 μl mucin sample or standard (0–1000 ng/ml) was added to each well and the plate was incubated at 37 °C for 40 min. It was incubated with 50 μl peroxidase conjugated DBA (1 mg/l) at 37 °C for another