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Participation of citral in the bronchodilatory effect of ginger oil and possible mechanism of action

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ABSTRACT

The extract of ginger, the rhizomes of *Zingiber officinale* Roscoe (Zingiberaceae), has been reported to possess anti-hyperactivity and anti-inflammation on airway. The present study described brochodilatory activity of ginger oil and identified its active compound. Ginger oil was extracted by hydro-distillation. The compositions of ginger oil were analyzed by gas chromatography and mass spectrometer. Citral, eucalyptol and camphene were found to be the major components. Ginger oil and citral, but not camphene, suppressed rat tracheal contraction induced by carbachol (CCh). Consistent with previous report, eucalyptol showed a relaxing effect on rat airway. Since the content of eucalyptol in ginger oil was relatively low, the contribution of eucalyptol to the bronchodilatory effect of ginger oil was small. To elucidate the mechanisms responsible for the myorelaxing effect, propranolol (a β-adrenergic receptor antagonist), indomethacin (a COX inhibitor) and L-NAME (a NOS inhibitor) were used to block the inhibitory effects of ginger oil and citral. It was found that propranolol, but not indomethacin and L-NAME, reversed bronchodilatory effects of both ginger oil and citral, suggesting that a possible mechanism involved β-adrenergic receptor. This study provides the pharmacological basis supporting the therapeutic potential of *Z. officinale* rhizomes as a bronchodilator.

1. Introduction

Ginger is a popular name of the rhizomes of *Zingiber officinale* Roscoe (Zingiberaceae) which is distributed worldwide particularly in Asia. In addition to being extensively used as spices in food and drinks, ginger has been employed as an alternative medicine around the world for treatment of gastrointestinal tract disorders, including dyspepsia, indigestion, diarrhea, motion sickness and nausea related to pregnancy, surgery or chemotherapy [1–3]. Recently, ginger has received increasing attention due to its pharmacological activities such as blood pressure lowering, pain-killing and uterine smooth muscle relaxing effects [4–6]. It has been reported that ginger reduced the symptoms, such as nocturnal cough and dyspnea in asthmatic patients [7]. Its hydroethanolic extract has been shown to inhibit airway hyperreactivity and lung inflammation [8,9]. The inhibitory effects of aqueous and methanolic crude extracts of ginger on tracheal smooth muscle were described [10]; however, the effects of ginger oil and its active compounds on airway smooth muscle have never been reported. The aim of this study is to investigate the effect of ginger oil on tracheal smooth muscle in vitro and to identify the compounds responsible for this activity. The possible mechanisms of action were also elucidated.

2. Materials and methods

2.1. Chemicals

Citral (*cis*/*trans*), eucalyptol, camphene, carbachol, propranolol, L-NAME and indomethacin were purchased from

Abbreviations: CCh, carbachol; COX, cyclooxygenase; GC/MS, gas chromatography and mass spectrometry; L-NAME, N_{ω}-nitro-L-arginine methyl ester; NOS, nitric oxide synthase.

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Sigma-Aldrich (USA). NaCl, $NaHCO_3$, $CaCl_2$, KCl, NaH_2PO_4, $MgSO_4$ and glucose were purchased from Carlo Erba (Italy).

2.2. Plant material and oil extract

Fresh rhizomes of *Z. officinale* (at 10 months of age) were collected from local markets in Muang district, Nakhon Ratchasima province, Thailand. The plant sample was identified at the Forest Herbarium of Thailand and confirmed by Dr. Surapon Saensouk, a plant taxonomist from Mahasarakham University. The specimen has been kept at the School of Biology, Institute of Science, Suranaree University of Technology. The voucher specimen number is Pharm-Chu-004.

Prior to the distillation procedure, unpeeled rhizomes were washed and chopped into small pieces. The sample was put into extract apparatus and subjected to hydro-distillation for 8 h at 80 °C. The obtained ginger oil was dried over anhydrous sodium sulfate and then kept in tightly sealed containers at 4 °C for subsequent experiments. The yield of ginger oil was 0.13% (w/w). A stock solution was obtained by dissolving small aliquots of ginger oil in hexane (1:1 v/v).

2.3. Determination of constituents of ginger oil by GC/MS

Ginger oil was analyzed chemically by gas chromatography and mass spectrometry (GC/MS; Model CP-3800-1200L Quadrupole MS/MS). Briefly, compounds were separated on a FactorFour capillary column VF-5ms (30 m × 2.25 mm; 0.25 μm) using the carrier gas helium (0.7 ml/min). The injector temperature was 250 °C and the column temperature was maintained at 40 °C for 5 min and then programmed at 4 °C/min to 250 °C. The spectrometers were operated in electron-ionization (EI) mode at 70 eV ionization energy, the scan range was 35–400 amu. The detector was set as fixed voltage at 1200 V and the scan rate was 0.5 s per scan. The ionization source temperature was 250 °C. The identification of the major compounds was performed by comparing their mass spectra with the NIST library available in the instrument and confirmed by comparing with standards.

2.4. Rat tracheal preparation and contractility measurements in vitro

Male Wistar rats, weighing 230–380 g, were used in this study. The experiments performed on rats were conducted in accordance with the current ethical principles and guidelines for the care and use of research animals of the Institutional Animal Care and Use Committee, National Research Council of Thailand, under our local institutional approval.

Rat trachea was prepared according to method previously described [11]. Rats were sacrificed by cervical dislocation. The trachea was carefully dissected and cleaned of external connective tissue and then cut into several two-cartilage segments. Each tracheal segment was mounted vertically in an organ bath (10 ml capacity) containing Krebs solution (37 °C pH 7.4) and aerated with carbogen (O_2 95% and CO_2 5%). The compositions of Krebs solution (mM) were: NaCl 120; $NaHCO_3$ 22; $CaCl_2$ 2.5; KCl 4.6; NaH_2PO_4 1.2; $MgSO_4$ 1.2 and glucose 11.5 (pH 7.4). One end of the tracheal ring was tied by a fine cotton thread to a hook at the bottom of the bath and the other to a force transducer (Force Transducer Model MLT 1030/D, ADI Instruments). Tissues were mounted under an initial resting tension of 1 g and left to equilibrate for a period of 30 min before starting the experimental protocol. Tension changes were recorded using isometric force transducers connected to a Power Lab Chart recorder (Model ML866, ADI Instruments). After the equilibration period, control contractions were induced by adding 1 μM carbachol (CCh), a non-selective muscarinic receptor agonist, to the organ bath. When two successive control contractions showed similar amplitude, preparations were considered to be equilibrated. The CCh-induced contraction was used as reference for maximal percentage response. The contractile amplitude was measured at the peak deflection.

2.5. Study on the effect of ginger oil on CCh-induced contraction

A tracheal segment was incubated in Krebs solution and equilibrated for 30 min. Tracheal contractions were induced by CCh (1 μM). Once sustained contractions elicited by CCh were established, ginger oil (22.5–1125 μg/ml final concentration) was added to obtain the dose response curve. The responses were expressed as percent reduction of the CCh-induced tracheal contraction. Control experiments were performed with the vehicle (0.125% hexane) which showed no effect on tracheal contraction.

2.6. Determination of active ingredients of ginger oil on the relaxing effect

To examine the compounds responsible for the myorelaxant effect of ginger oil, tracheal preparations were treated with ginger oil (225 μg/ml) and its major constituents, namely citral (140 μg/ml), eucalyptol (15 μg/ml) and camphene (10 μg/ml) for 10 min after a stable response to CCh-induced contraction were achieved. Reduction of contraction was recorded during the last 5-min period. In the parallel control experiments, the effects of the solvent vehicle on the contraction of the trachea were also tested.

2.7. Study on the effect of β-adrenergic antagonist, NOS and COX inhibitors on the relaxation produced by ginger oil and citral

To examine the possible mechanism of tracheal relaxant effect of ginger oil, the tracheal strips were pretreated with 3 μM propranolol (a β-adrenergic blocker), 100 μM L-NAME (a nitric oxide synthase (NOS) inhibitor) and10 μM indomethacin (a cyclooxygenase (COX) inhibitor) prior to CCh for an appropriate period of time in accordance with previous reports [12–14]. After sustained CCh-induced contraction, ginger oil or citral was added into the organ bath. Changes of contraction force were recorded over the same period as described in the previous section.

2.8. Statistical analysis

Data are expressed as mean ± standard error of mean (S.E.M). Maximal contraction after each concentration of ginger oil was used to construct a concentration–response curve. It was expressed as the percentage of control contraction evoked by CCh. The EC_{50}, defined as the concentration (in μg/ml) of ginger oil at which the maximal CCh-induced contraction was