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Inhibition effects of Moutan Cortex Radicis on secretion of eotaxin in A549 human epithelial cells and eosinophil migration

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Abstract

Eosinophils have been implicated in a broad range of diseases, most notably allergic conditions (e.g. asthma, rhinitis and atopic dermatitis) and inflammatory diseases. These diseases are characterized by an accumulation of eosinophils in the tissue. Defining the mechanisms that control eosinophil recruitment is fundamental to understanding how these diseases progress and may identify a novel target for drug therapy. Eotaxin is a potent eosinophil-specific chemokine that is released in the respiratory epithelium after allergic stimulation.

Aim of the study: In this study, we determined whether Moutan Cortex Radicis (MCR), a plant extract, effects eotaxin secretion from A549 epithelial cells and eosinophil chemotaxis, and then examined the mechanism involved.

Materials and methods: Prior to assaying MCR's effects, A549 cells were stimulated with tumor necrosis factor-α (TNF-α), interleukin-4 (IL-4) and IL-1β to induce expression of chemokines and adhesion molecules involved in eosinophil chemotaxis. In the presence of MCR, eotaxin, regulated on activation in normal T cells expressed and secreted (RANTES), IL-8, IL-16, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) transcripts were quantitated by real-time RT-PCR.

Results: As a result, 0.01, 1, and 100 μg/ml of MCR treatments reduced eotaxin expression significantly and 0.01, 0.1, 1, 10, and 100 μg/ml of MCR reduced significantly eotaxin secretion. In addition, MCR treatment significantly inhibited eosinophil migration toward A549 medium. And 100 μg/ml of MCR suppressed the activated of nuclear factor (NF)-κB.

Conclusions: These findings indicate that suppressed eotaxin secretion by MCR treatment is due to the inhibition of NF-κB activation. Therefore, MCR might be of therapeutic value in treating asthma.

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Keywords: Moutan Cortex Radicis; Eosinophil; Eotaxin; Chemotaxis; NF-κB

1. Introduction

The role of inflammation in asthma and other airway allergic diseases is widely appreciated; hence, airway inflammation is now a defining feature of asthma (Kay, 1991). Eosinophil accumulation is also an important factor in allergic airway disease. This is based on the knowledge that eosinophils are present in allergic airways, and on the correlation between a loss of asthma symptoms and a loss of eosinophil accumulation in the airway (Djukanovic et al., 1992).

Eosinophils express several membrane receptors. Among them are receptors for immunoglobulins, cytokines, chemokines such as eotaxin and regulated on activation in normal T cells expressed and secreted (RANTES) and adhesion receptors, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (Bentley et al., 1993). Some pro-inflammatory mediators released from eosinophils may cause tissue damage and induce physiological

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abnormalities. Local chemokine concentration increases are facilitated by chemokine-glycosaminoglycan interactions, signaling cell migration and trafficking inflammation (Halden et al., 2004). Eotaxin is an eosinophil-specific chemoattractant. This specificity has been demonstrated in both mouse (Gonzalo et al., 1996) and human (Ponath et al., 1996). Natural products capable of inhibiting eosinophil function and/or infiltration are desirable treatments for asthma and other allergic diseases (Sa-Nunes et al., 2006) and Li reported that Achyranthes bidentata blume enhance the apoptosis of eosinophils (Li et al., 2003).

Many previous reports suggested that activated NF-κB may have be a crucial role for increased expression of many inflammatory genes of airway inflammation in asthma (Matsukura et al., 1999; Jedrzkiewicz et al., 2000; Wong et al., 2002; Wong et al., 2005) and regulation of eosinphil (Ward et al., 1999).

Moutan Cortex Radicis (MCR), the root cortex of *Paeonia suffruticosa* Andrews (Ranunculaceae), is a medicinal plant widely used as an analgesic, antispasmodic and anti-inflammatory agent. The drug has also been used for gynaecological disease, and to relieve neuropathic pain. MCR reportedly inhibits IL-8 and MCP-1 secretions (Oh et al., 2003; Tatsumi et al., 2004), and reactive oxygen species production (Rho et al., 2005).

In this study, we first confirmed the effect of MCR on the accumulation of messenger RNA (mRNA) induced by tumor necrosis factor-α (TNF-α), interleukin-4 (IL-4) and IL-1β in A549 cells. Second, we assayed MCR's inhibition of eotaxin at the protein level. Third, we found that suppressing eotaxin inhibits eosinophil migration, and fourth, we investigated that the effect of MCR is by the modulation of NF-κB activity on the regulation of eotaxin in stimulated A549 cells.

2. Materials and methods

2.1. Cell culture

A549 cells, human type II-like epithelial lung cells, were obtained from the Korean Cell Line Bank (Cancer Research Institute, Seoul, Korea). These cells were cultured in tissue flasks in 100% humidity and 5% CO_2 at 37 °C in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum and penicillin–streptomycin, at 1×10^6 cells/ml. A549 cells were then plated onto 12-well, flat-bottom tissue culture plates at a density of 5×10^5 cells/well in hormonally defined RPMI media as described previously. The medium was changed every 2 days until the cells became confluent at which point they were used for experiments.

2.2. Preparation of MCR

MCR was purchased from the Sun Ten Pharmaceutical (Taipei, Taiwan). MCR was powdered to 0.1 g and extracted with 10 ml DW using stirrer for overnight at room temperature. The sample was centrifuged for 10 min at 3000 rpm. The supernatant was sterilized by passing through 0.22 μm syringe filter. Then the stock of MCR was used for the experiments.

2.3. High-performance liquid chromatography (HPLC) analysis of MCR

MCR powder was accurately weighed to 1 g and dissolved in 10 ml of 50% methanol. The sample was ultrasonic for 15 min and centrifuged at 15,000 rpm for 10 min. The supernatant of the sample was filtered through a 0.45 μm syringe filter. The paeonol in the MCR was quantitatively analyzed by Korean Pharmacopoeia's method. A marker substance, paeonol (Wako Pure Chemicals Industries Co., Osaka, Japan), was used for quantitative comparison to MCR. An amount of 1.0 mg of Paeonol was dissolved in 1.0 ml methanol and diluted to 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0 mg/ml. A standard HPLC chromatogram was obtained. The relationship between the concentration and the peak-area was measured by minimum square method (R^2 value).

The quantity of paeonol in MCR was calculated using the following formula:

$$\text{mg of paeonol} = \{\text{mg of paeonol} \times \text{AT}/\text{AS}\}/n, \quad (n = 3)$$

where AT is the peak area of the test sample, and AS is the peak area of the standard.

HPLC analysis was carried out with a Waters Breeze System (717+ Autosampler, 2996 photodiode array system, 1525 binary HPLC pump, TCM (oven column), Waters Co., Milford, USA) and a Waters Empower System (Ver. 5.00, Waters Co., Milford, USA).

The test sample was analyzed by reverse-phase HPLC on a C18 column (Nova-Pak, 150 mm × 3.9 mm (i.d.), Waters Co., Milford, USA) under isocratic conditions with acetonitrile–water with 2% glacial acetic acid (1:1, v/v) at 1 ml/min, UV detection at 274 nm, 10 μl injections and a column temperature of 25 °C.

2.4. Cytotoxicity assays

To determine the cytotoxicity capacity of the A549 cells, the Cell Titer 96 non-radioactive cell proliferation assay (Promega, Medison, USA) was used following the directions provided by the manufacturer. Cells were plated at 2×10^4 cells/well in a total volume of 100 μl. Cells were then washed two times and cultured in MCR extract (0, 0.1, 1, 10, 100, and 1000 μg/ml) containing medium. Cells were incubated for 48 h at 37 °C in 5% CO_2 incubator.

2.5. Cytokine assays

Cells were plated at 5×10^5 cells/well in 1 ml of medium. Upon reaching confluency, the medium was changed to serum-free RPMI for 24 h. Cells were stimulated with 100 ng/ml TNF-α (Biosource, Camarillo, USA) and 100 ng/ml IL-4 (Biosource, Camarillo, USA) to stimulate eotaxin, and incubated for 3 h. Next, the cells were stimulated with 100 ng/ml TNF-α and 10 ng/ml IL-1β (Biosource, Camarillo, USA) combined to stimulate RANTES. After 48-h incubation, the A549 cells were washed twice with PBS and cultured in MCR extract (0.01, 1 or 100 μg/ml) in medium. Cells were incubated for 48 h. Cell

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