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Inhibitory effects of Schizandrae Fructus on eotaxin secretion in A549 human epithelial cells and eosinophil migration

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Abstract

Eosinophilia 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 affected tissue. Defining the mechanisms that control the recruitment of eosinophil is fundamental to understanding how these diseases progress and identifying a novel target for drug therapy. Accordingly, this study was conducted to evaluate the regulatory effect of Schizandrae Fructus (SF) on the expression of eotaxin, an eosinophil-specific chemokine released in respiratory epithelium following allergic stimulation, as well as its effects on eosinophil migration.

To accomplish this, human epithelial lung cells (A549 cell) were stimulated with a combination of TNF-α (100 ng/ml) and IL-4 (100 ng/ml) for 24 h. The cells were then restimulated with TNF-α (100 ng/ml) and IL-1β (10 ng/ml) to induce the expression of chemokines and adhesion molecules involved in eosinophil chemotaxis for another 24 h. Next, the samples were treated with various concentrations of Schizandrae Fructus (SF) (1, 10, 100, 1000 μg/ml) or one of the major constituents of SF, schizandrin (0.1, 1, 10, 100 μg/ml), after which following inhibition effect assay was performed triplicates in three independence.

The levels of eotaxin in secreted proteins were suppressed significantly by SF (100 and 1000 μg/ml, $p<0.01$) and schizandrin (10 and 100 μg/ml, $p<0.01$). In addition, SF (1, 10, 100 and 1000 μg/ml) decreased mRNA expression levels in A549 cells significantly ($p<0.01$). Eosinophil recruitment to lung epithelial cells was also reduced by SF, which indicates that eotaxin plays a role in eosinophil recruitment. Furthermore, treatment with SF suppressed the expression of another chemokine, IL-8 (0.1 and 1 μg/ml SF, $p<0.01$), as well as intercellular adhesion molecule-1 (10 and 100 μg/ml SF, $p<0.01$) and vascular cell adhesion molecule-1 (0.1 and 1 μg/ml SF, $p<0.05$), which are all related

Abbreviations: ERK, extracellular signal-regulated kinases p42/p44; HOG, p38/MAPK; HPLC, high-performance liquid chromatography; ICAM-1, intercellular adhesion molecule-1; IL-4, interleukin-4; JNK/SAPK, c-Jun N-terminal kinase/stress-activated protein kinase; MAPK, mitogen-activated protein kinases; MTS, [3-(4; 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; SF, Schizandrae Fructus; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1.

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to eosinophil migration. Taken together, these findings indicate that SF may be a desirable medicinal plant for the treatment of allergic diseases.

Keywords: Schizandrae Fructus; Eosinophil migration; Eotaxin; Allergic diseases

Introduction

Because exposure to environmental hazards is inevitable, outbreaks of allergic diseases such as allergic asthma, atopic dermatitis, and allergic rhinitis have increased. Indeed, in the United States more than 50 million people suffer from allergic diseases each year, costing the US health care system approximately $18 billion annually (Elsner et al. 2004). As a result, many academic and industrial studies have been conducted to define disease mechanisms and develop therapies to treat or prevent the symptoms of allergies.

The initial stage of asthmatic symptoms is airway inflammation, in which eosinophils play a crucial role (Kay 1991). Eosinophils are present in excess in the airways of asthma patients; however, their accumulation decreases with subsidence of the symptoms of asthma. During an asthma attack, eosinophils selectively migrate and adhere to vascular endothelial cells, after which they migrate into the airways in response to chemokine recruitment. Once in the airway, they infiltrate and cause inflammation (Djukanovic et al. 1992).

Many natural products used in traditional oriental medicine are reportedly good agents for the treatment of asthma (Lima-Landman et al. 2007). For example, it has been suggested that Moutan Cortex Radicis reduced eotaxin secretion (Kim et al. 2007). However, despite their remarkable ability to treat asthma, most natural products have not been widely used in western societies, because little is known about the modes of action at the molecular level. One such product, Schizandrae Fructus (SF) is the fruit of *Schizandra chinensis* Baill. SF, which is an oriental herb that contains schizandrin as one of its major constituents, is used by traditional oriental clinicians to treat several diseases including hepatitis (Liu 1989; Liu and Lesca 1982), and cancer (Li 1991). Accordingly, there have been several studies conducted to evaluate the molecular mechanisms responsible for the anti-tumor effects (Huang et al. 2004), effects on cycloheximide-induced amnesia (Hsieh et al. 1999), and inhibitory effects on human articular cartilage and chondrocytes (Choi et al. 2006a) that are exerted by SF. However, few studies have been conducted to evaluate the effects of SF on airway-related diseases. Therefore, we evaluated the effects of SF on asthma while focusing on its ability to recruit eosinophils.

Materials and methods

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 100 mm tissue culture plates (Corning, Corning, NY, USA) in RPMI medium (Invitrogen, Rockville, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, Logan, UT, USA) and 100 U/ml penicillin–streptomycin (Invitrogen, Rockville, MD, USA) at a density of 1×10^6 cells/ml. The plates were incubated at 37 °C under 100% humidity and 5% CO_2. The cells were sub-cultured every 3–4 days to maintain a density of 1×10^6 cells/ml.

Preparation of SF and schizandrin

SF powder granules were purchased from Sun Ten Pharmaceutical (Taipei, Taiwan) and schizandrin was purchased from Wako Pure Chemical Industries, Ltd., Japan. One hundred mg of SF powder was added to 10 ml of DW, while 1 mg of schizandrin was added to 1 ml of DW. The mixtures were then stirred overnight at room temperature. Each sample was then centrifuged for 10 min at 3000 rpm (Eppendorf, Hamburg, Germany), after which the supernatant was removed and sterilized by passing it through a 0.22 μm syringe filter. The stocks of SF or schizandrin were then diluted to various concentrations and used in the subsequent experiments.

Endotoxin test (LAL test)

Endotoxin toxicity was determined using a quantitative, Limulus Amebocyte Lysate (LAL) QCL-1000 test (CAMBREX, Harriman, NY, USA) using the microplate method described by the manufacturer. The kit included vials of standard *E. coli* O111:B4 containing a defined number of endotoxin units (EU). A standard curve ranging from 0 to 1.0 EU/ml was constructed by plotting the OD405 nm versus EU per absorbance unit. SF was also assayed in duplicate and the concentration was plotted against OD405 nm. The absorbance of p-nitroaniline released from the substrate was measured