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ORIGINAL ARTICLE

Caffeic acid phenethyl ester suppresses eotaxin secretion and nuclear p-STAT6 in human lung fibroblast cells

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Caffeic acid phenethyl ester;
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Background: Caffeic acid phenethyl ester (CAPE), an active component of propolis, has been proven to have anti-inflammatory and antiallergic properties. We have investigated the activity of CAPE in regulating cytokine-induced eotaxin production and its related signal protein, signal transducer and activator of transcription 6 (STAT6), in human lung fibroblast.
Methods: The CCD-11Lu human lung fibroblast cell line was used as an *in vitro* model. Cells were pretreated with CAPE followed by stimulation with interleukin-4 and tumor necrosis factor alpha. The levels of eotaxin in cultured supernatants were measured by enzyme-linked immunosorbent assay. The amounts of STAT6 and phosphorylated STAT6 in cellular nuclear protein extracts were determined by Western blot analysis. STAT6 DNA binding activities were detected by electrophoretic mobility shift assay.
Results: Pretreated CCD-11Lu cells with noncytotoxic doses (0.1—10 μM) of CAPE inhibited the production of eotaxin under stimulation of interleukin-4 (10 ng/mL) and tumor necrosis factor alpha (10 ng/mL). CAPE pretreatment also decreased the amount of phosphorylated STAT6 and the STAT6 DNA binding complexes in nuclear extracts.
Conclusion: CAPE inhibited the production of eotaxin protein in stimulated human lung fibroblast cells in a dose-dependent manner. This activity is, at least, through STAT6 inhibition. We suggest that CAPE is a promising agent in controlling eotaxin secretion and subsequent

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eosinophils influx and may therefore have a potential role to play in treating allergic airway disease.

Introduction

Asthma is a chronic inflammatory condition of the airways resulting in episodic airflow obstruction, airway hyperresponsiveness, and airway remodeling. Asthmatic airway tissues have increased numbers of activated eosinophils, neutrophils, mast cells, and activated lymphocytes, especially Type 2 T helper (Th2) cells.[1–3] Massive accumulation of eosinophils in airways is a fundamental trait of bronchial asthma.[4] Eosinophils and their products, including cytotoxic granule proteins and *de novo* synthesized leukotrienes, are important effectors in the pathophysiology of airway allergic inflammation, causing destruction of airway epithelium, sensitization of airway nerve terminals, vascular leakage, and other pathological changes.[5] Th2 lymphocytes and their products, such as interleukin (IL)-4, IL-5, and IL-13, mediated the airway eosinophil infiltration and IgE production,[6] and are known to stimulate the expression of several chemokines and mediators, such as eotaxin.[7,8] These chemokines may favor the recruitment of Th2 cells, eosinophils, basophils, and mast cells into the airway,[9] which lead to chronic airway inflammation.

Eotaxin, a potent chemokine for eosinophil, was originally discovered in 1994, has an important local role in the recruitment of eosinophils from the microvasculature into the tissue at sites of local allergic inflammation.[10–12] Eotaxin can be produced by many cell types, including lung fibroblast, smooth muscle cell, endothelial cell, alveolar macrophage, eosinophil, lymphocyte, and bronchial epithelial cell.[13] Among them, fibroblast is the major cellular source of eotaxin.[14,15] Several studies revealed that the combination of IL-4 and tumor necrosis factor alpha (TNF-α) could amplify the production of eotaxin in human lung fibroblast, airway epithelial cell, smooth muscle cell, and dermal fibroblast.[7,9,16–19] The mechanism operative in regulating IL-4 and TNF-α-stimulated eotaxin expression in epithelial cell and human lung fibroblast was mediated by activation of transcription factors, NF-kappa B (NF-κB) and signal transducer and activator of transcription 6 (STAT6).[16,17]

Caffeic acid phenethyl ester (CAPE) is a bioactive component of propolis (honeybee resin), which has several biological properties including anti-inflammatory, antioxidant, antimicrobial, and antitumor activities.[20] More recently, in murine model of ovalbumin-induced asthma, CAPE had significant effects on inhibition of characteristic asthmatic reactions (inflammatory cells; cytokines IL-4, IL-5, and TNF-α; mucus hypersecretion; and serum ovalbumin-specific IgE), diminished reactive oxygen species in bronchoalveolar fluid, and NF-κB DNA binding activity.[21] Although the eotaxin promoter contains overlapping consensus bindings sites for transcription factors, NF-κB and STAT6 are known to mediate responses to IL-4 and TNF-α.[17] Data showed that both IL-4 and TNF-α require STAT6 as mediator to activate eotaxin gene expression in fibroblast cells.[19]

In our previous study,[22] we demonstrated that CAPE can inhibit eotaxin secretion in human lung fibroblast (CCD-11Lu cell line) stimulated synergistically by IL-13 and TNF-α. We also showed that CAPE was able to inhibit NF-κB activation. The aim of present study is to check if CAPE possesses inhibitory effect on STAT6 activation.

Methods

Reagents

CAPE was purchased from Calbiochem (San Diego, CA, USA) and dissolved in dimethyl sulfoxide (Merck, Darmstadt, Germany) in a stock concentration of 10 mM. Recombinant human IL-4 and TNF-α were purchased from Peprotech (Rocky Hill, NJ, USA). Eotaxin sandwich ELISA kits were purchased from BD biosciences (San Jose, CA, USA).

Cells and culture medium

The human lung fibroblast cell line, CCD-11Lu, was purchased from American Type Culture Collection (Manassas, VA, USA). The cells were cultured in minimum essential medium (Gibco-BRL, Rockville, MD, USA) containing 10% heat-inactivated fetal bovine serum, 100 units/mL of penicillin, and 100 μg/mL of streptomycin (Invitrogen, Carlsbad, CA, USA) in a humidified 5% carbon dioxide/95% air atmosphere at 37°C.

Cell culture and study design

We cultured cells under the following conditions:

1. Cells (1×10^5cells/well) were seeded in each well of 12-well plates (BD biosciences, San Jose, CA, USA) to settle and adhere for 24 hours. The culture medium was removed and replaced by serum-free medium, then the confluent monolayer were incubated with recombinant human IL-4 (10 ng/mL, 50 ng/mL), TNF-α (10 ng/mL, 50 ng/mL), or the combination of different concentration in the dose range 10 ng/mL and 50 ng/mL for 24 hours. Cells without stimulation were used as negative control. The supernatants were harvested and conserved at −80°C.
2. We pretreated cell under condition (1) with CAPE of various concentration (0.1 μM, 1 μM, 10 μM) for 3 hours (which is the earliest effective time of CAPE on eotaxin production in our preliminary study), followed by the stimulation with recombinant human IL-4 (10 ng/mL) and TNF-α (10 ng/mL) for another 24 hours. The supernatants were harvested and conserved at −80°C.