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ORIGINAL ARTICLE

The Immunoregulatory Effects of Caffeic Acid Phenethyl Ester on the Cytokine Secretion of Peripheral Blood Mononuclear Cells From Asthmatic Children

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Key Words
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Background: Asthma is a chronic inflammatory disease of the airways for which current treatments are mainly based on pharmacological interventions, such as glucocorticoid therapy. Our objective was to study the immunoregulatory effects of caffeic acid phenethyl ester (CAPE, a phytochemical synthesized from propolis) on cytokine secretion of peripheral blood mononuclear cells (PBMCs) from asthmatic children.
Methods: PBMCs from asthmatic children (5.5 ± 3.3 years old, $n = 28$) and healthy children (5.6 ± 2.8 years old, $n = 23$) were co-cultured with CAPE *in vitro* with and without phorbol-12-myristate-13-acetate-ionomycin.
Results: Our results show that predominant interleukin 4 (IL-4) and interferon-gamma secretion of cultured supernatant were detected in healthy donors compared with asthmatics. In the presence of phorbol-12-myristate-13-acetate-ionomycin, with or without CAPE treatment, the asthmatic children showed significantly decreased levels of IL-10 secretion compared with the healthy controls. However, CAPE significantly decreased IL-10 and interferon-gamma in healthy donors. There was a slight but not statistically significant reduction of IL-4 secretion in CAPE-treated PBMCs compared with untreated control PBMCs from the healthy children. Our data also shows that CAPE significantly enhanced transforming growth factor-beta 1 production from PBMCs from asthmatic children.

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Conclusion: The immunoregulatory effects of CAPE on human PBMCs may be through the induction of regulatory T cells, as evidenced by the enhanced transforming growth factor-beta 1 production from PBMCs from asthmatic children in our study.

1. Introduction

Caffeic acid phenethyl ester (CAPE) is a phytochemical synthesized from honeybee propolis,[1] a naturally occurring bee product. Several studies have shown that CAPE has antitumor,[2–6] anti-inflammatory,[7,8] and antioxidant properties.[9–12] CAPE downregulates mitogen-induced T-cell proliferation and lymphokine production[13] by inhibition of DNA binding and transcription of nuclear factor-κappa B and the nuclear factor of activated T cells in stimulated T-cells.[14] *In vivo,* the immunomodulatory effects of CAPE have also been observed in CAPE-administered mice, with increased $CD4^+$ T cell subsets and enhanced anti-CD3-induced cytokine production in splenocytes.[15] However, there are no studies to date on how CAPE might affect the immune response in asthma.

Asthma is a chronic inflammatory disease of the airways caused by aberrant T-helper 2 (TH2) immune responses. Activation of TH2 cells mediate the synthesis of immunoglobulin E via interleukin-4 (IL-4) and eosinophilic inflammation via IL-5, which, together with IL-13, contributes to airway hyper-responsiveness and other clinical features of allergic disease.[16] Current pharmacotherapy for asthma includes glucocorticoids for relief of wheezing in preschool children.[17] Although glucocorticoids can effectively control attacks of asthma, many patients develop side effects as a result of long-term steroid therapy.[18] Even inhaled glucocorticoids have been shown to suppress bone growth in pediatric patients.[19] Thus, there is a need for novel therapeutic strategies, which are equally effective but with minimal side effects. This study investigated the immunoregulatory effects of CAPE *in vitro* on cytokine secretion of the peripheral blood mononuclear cells (PBMCs) isolated from both asthmatic and healthy children.

2. Materials and Methods

2.1. Participants

Heparinized blood was obtained from 28 untreated asthmatic patients (11 girls and 17 boys) and 23 healthy nonallergic controls (9 girls and 14 boys). Average age was 5.5 ± 3.3 years for asthmatic patients and 5.6 ± 2.8 years for healthy controls. Our asthmatic patients, children with mild intermittent asthma who were not yet treated with corticosteroids and/or bronchodilators, were recruited from the Department of Pediatrics, Cardinal Tien Hospital (Taipei, Taiwan). Healthy children, defined as those without symptoms of allergic or infectious diseases, were enlisted from participants at a promotional program hosted by Cardinal Tien Hospital on December 18, 2004. Total serum immunoglobulin E concentrations (analyzed by chemiluminescence method) in asthmatic and healthy children were 659.6 ± 648.2 kU/L and 32.0 ± 22.8 kU/L, respectively. The study protocol was reviewed and approved by the Committee on Human Experiments of Catholic Cardinal Tien Hospital, Taipei, Taiwan. Written informed consent was obtained from each participant's parents.

2.2. Reagents

CAPE was purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Phorbol 12-myristate 13-acetate (PMA)-ionomycin was all purchased from Sigma (Germany).

2.3. Preparation of PBMCs

Human PBMCs were isolated from heparinized blood after centrifugation in Ficoll-Hypaque (Pharmacia, Sweden) density gradient. The PBMC layers were collected and washed twice with cold Hanks' buffer saline solution (HyClone, USA). The cells were then resuspended in complete medium (Roswell Park Memorial Institute 1640 media supplemented with 10% fetal bovine serum, 2mM L-glutamine, 10mM HEPES, 100 U/mL penicillin, 100 U/mL streptomycin, and 5.78×10^{-5}M β-mercaptoethanol, all purchased from HyClone, USA).

2.4. Quantification of cytokine secretion by enzyme-linked immunosorbent assay

PBMC (2×10^6 cells/mL) were stimulated with 20 ng/mL PMA, Sigma (Germany) and 1μM ionomycin (Sigma, Germany) in the presence or absence of 2.5 μg/mL CAPE, and supernatants were collected after 24 hours. Culture supernatants were spun free of cells and aliquots were frozen at −80°C. Levels of transforming growth factor beta 1 (TGF-β1) in acidified supernatants were determined by capture enzyme-linked immunosorbent assay according to manufacturer's instructions (R&D Systems, Germany). Levels of IL-4, IL-5, IL-10, and interferon-gamma (IFN-γ) were measured by capture ELISA (BD PharMingen, USA) according to manufacturer's instructions.

2.5. Statistical analysis

Two-way analysis of variance was used to test for statistically significant differences; p values less than 0.05 were considered significant. All cultures were done in triplicate, and standard deviations are represented by error bars.