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Caffeic acid phenethyl ester lessens disease symptoms in an experimental autoimmune uveoretinitis mouse model

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ABSTRACT

Experimental autoimmune uveoretinitis (EAU) is an autoimmune disease that models human uveitis. Caffeic acid phenethyl ester (CAPE), a phenolic compound isolated from propolis, possesses anti-inflammatory and immunomodulatory properties. CAPE demonstrates therapeutic potential in several animal disease models through its ability to inhibit NF-κB activity. To evaluate these therapeutic effects in EAU, we administered CAPE in a model of EAU that develops after immunization with interphotoreceptor retinal-binding protein (IRBP) in B10.RIII and C57BL/6 mice. Importantly, we found that CAPE lessened the severity of EAU symptoms in both mouse strains. Notably, treated mice exhibited a decrease in the ocular infiltration of immune cell populations into the retina; reduced TNF-α, IL-6, and IFN-γ serum levels: and inhibited TNF-α mRNA expression in retinal tissues. Although CAPE failed to inhibit IRBP-specific T cell proliferation, it was sufficient to suppress cytokine, chemokine, and IRBP-specific antibody production. In addition, retinal tissues isolated from CAPE-treated EAU mice revealed a decrease in NF-κB p65 and phospho-IκBα. The data identify CAPE as a potential therapeutic agent for autoimmune uveitis that acts by inhibiting cellular infiltration into the retina, reducing the levels of pro-inflammatory cytokines, chemokine, and IRBP-specific antibody and blocking NF-κB pathway activation.

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1. Introduction

Human uveitis is an ocular-inflammatory disease that can often lead to blindness. Experimental autoimmune uveoretinitis (EAU) is an animal model that shares many features with human uveitic disorders (Caspi et al., 1988; Nussenblatt, 1991). EAU is an organ-specific, T cell-mediated autoimmune disease that can be induced by immunization with retinal antigens (Ags), including retinal S–Ag and interphotoreceptor retinal-binding protein (IRBP), or by the adoptive transfer of retinal Ag-specific T lymphocytes (Caspi et al., 1986; Gregerson et al., 1986; Mochizuki et al., 1985). EAU is considered to be a predominantly Th1-mediated autoimmune disease. As such, it is thought that the inhibition of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) could mitigate EAU symptoms (Caspi, 2002; Rizzo et al., 1996). Macrophages and neutrophils are known to be key mediators of tissue damage in several experimental autoimmune disease models, and in agreement, both macrophages and neutrophils are the major effectors of tissue damage in uveitis (Sonoda et al., 2003; Yamamoto et al., 2010). Nuclear factor-kappa B (NF-κB) is implicated in the pathogenesis of many autoimmune diseases, including rheumatoid arthritis, type I diabetes, and multiple sclerosis (Pai and Thomas, 2008). Indeed, it was reported that NF-κB attenuation could alleviate the symptoms of EAU following treatment with an NF-κB inhibitor (Kitamei et al., 2006).

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Polyphenolic compounds derived from natural products are well known to have many advantages, namely, anti-oxidant (Chan et al., 2000), anti-tumoral (Suganuma et al., 1999), and anti-inflammatory properties (Martinez and Moreno, 2000). Caffeic acid phenethyl ester (CAPE, empirical formula $C_{17}H_{16}O_4$, molecular weight 284.31 g mol^{-1}) is a phenolic compound isolated from the propolis of honeybee hives (Fig. 1) and has anti-viral, anti-tumoral, anti-inflammatory, and immunomodulatory properties (Gokalp et al., 2006; Ozer et al., 2005). Several investigators have demonstrated the therapeutic potential of CAPE in treating allergic asthma (Jung et al., 2008b) and experimental autoimmune encephalomyelitis (EAE)-induced oxidative stress (Ilhan et al., 2004) by inhibiting NF-κB activity. In addition, CAPE is a potent inhibitor of mitogen-induced T cell proliferation and lymphokine production (Ansorge et al., 2003), and can also induce leukocyte apoptosis to further modulate NF-κB activity (Orban et al., 2000). However, no reports have demonstrated the effects of CAPE treatment in a mouse model of EAU.

In the current study, we demonstrated the therapeutic effects of CAPE in C57BL/6 and B10.RIII mice with fully developed EAU and provide mechanistic evidence for its suppressive effect on EAU pathogenesis. Notably, CAPE reduced immune cell infiltration into the retina and hindered IRBP-specific T cell cytokine, chemokine, and antibody production in a proliferation-independent manner. In addition, CAPE sufficiently inhibited NF-κB pathway activation. As such, we believe our study demonstrates the therapeutic potential of CAPE in EAU therapy.

2. Materials and methods

2.1. Mice and reagents

B10.RIII mice (8- to 12-weeks old) were purchased from Jackson Laboratory (Bar Harbor, ME). Wild-type C57BL/6 mice (8- to 10-weeks old) were purchased from Orient Bio, Inc. (Sungnam, Korea). All mice were bred and maintained under specific pathogen-free conditions in the Animal Care Facility at the College of Medicine, Inje University. All experimental procedures were examined and approved by the Institutional Animal Care and Use Committee of Inje University (Protocol No. 2013-053).

Human IRBP peptide spanning amino acid residues 161–180 (IRBP161–180, SGIPYIISYLHPGNTILHVD) and human IRBP peptide 1–20 (IRBP1–20, GPTHLFQPSLVLDMAKVLLD) were synthesized by Peptron (Daejeon, Korea). Complete Freund's adjuvant (CFA) containing 1.0 mg/mL *Mycobacterium tuberculosis* was purchased from Sigma–Aldrich (St. Louis, MO). Purified *Bordetella pertussis* toxin (PTX) and CAPE were purchased from Sigma Chemical Co. (St. Louis, MO). CAPE was first dissolved in dimethylsulfoxide (DMSO) and then diluted in phosphate-buffered saline (PBS).

2.2. EAU induction and clinical evaluation

B10.RIII and C57BL/6 mice were immunized subcutaneously (s.c.) in the thighs and tail base with human IRBP peptides emulsified in CFA in a total volume of 200 μL. Specifically, B10.RIII and C57BL/6 mice were immunized with 50 μg of IRBP161–180 or 250 μg of IRBP1–20, respectively. An additional intraperitoneal (i.p.) injection of 0.2 μg of PTX was administered in 200 μL of PBS to C57BL/6 mice on post-immunization (PI) days 0 and 2.

Clinical evaluation of retinal inflammation was recorded every 2 or 3 days by funduscopic examination. The severity of EAU was graded from 0 to 4, as described previously (Caspi, 2003). The clinical scoring was based on vessel dilatation, number of white focal lesions in vessels, and extent of retinal vessel exudate, hemorrhage, and detachment.

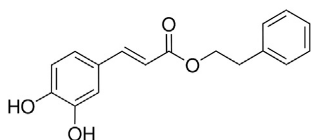

Fig. 1. Structure of caffeic acid phenethyl ester (2-cyclohexylethyl (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate).

2.3. CAPE treatment

Mice were injected i.p. with 10 mg/kg CAPE or vehicle (Pramanik et al., 2013). CAPE treatments were started on PI day 7, when EAU was developed (mean clinical score, ~1). B10.RIII mice were treated daily for 14 days, whereas C57BL/6 mice were treated for 10 or 14 days. Vehicle groups were treated with an equivalent volume of PBS.

2.4. Retinal flat mounts

Retinal flat mounts were prepared according to a described previously method (Li et al., 2011). Briefly, mice were anesthetized with an i.p. injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). The lateral canthus of the left orbit was chosen as the injection point. Gentle pressure was applied to the periorbital area with two fingers in order to expose the eye, and 100 μL of FITC-dextran (50 mg/mL in ultrapure water; Sigma–Aldrich) was injected. Enucleated eyes were then fixed in 4% paraformaldehyde for 30 min at room temperature. The cornea, iris, lens, and vitreous were gently removed under a stereomicroscope (S6E; Leica, Wetzlar, Germany). Four radial incisions were made in the dissected retina, and it was flattened with a coverslip.

2.5. Histopathology

Mouse eyes were collected on PI day 21, fixed in 10% buffered formalin solution (Sigma), and embedded in paraffin. Sections (6–8 μm) were prepared and stained with hematoxylin and eosin. The histological findings (original magnification, × 100 and × 200) were examined for inflammatory infiltration as well as retinal folding and detachment.

2.6. Flow cytometry

For staining, cell samples were prepared from the retinas of EAU-induced mice on PI days 7, 14, and 21. Cells were incubated with the Fc blocker 2.4G2 for 5 min and stained with 0.5 μg conjugated antibodies as follows: CD45-APC (clone 104), and CD3-FITC (clone 145-2C11) for T cells; CD45-APC, CD11b-PE (clone M1/70), and F4/80-PE/Cy5 (clone BM8) for macrophages; and CD45-APC, CD11b-PE, and Ly6G-PE/Cy7 (clone 1A8) for neutrophils. FACS data were acquired on a FACS Canto II flow cytometer (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (TreeStar; Ashland, OR). All antibodies were obtained from eBiosciences, Inc. (San Diego, CA).

2.7. T cell proliferation assay

For proliferation assays, cells isolated from the spleen and draining lymph nodes (DLN; the inguinal and iliac lymph nodes) were labeled with CFSE for 5 min at 37 °C (Choi et al., 2006). Cells were washed twice with PBS, and then stimulated with IRBP peptide (1, 10, 20, or 50 μg/mL) or concanavalin A (Con A; 5 μg/mL,

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