

ORIGINAL ARTICLE

Caffeic acid phenethyl ester promotes anti-inflammatory effects by inhibiting MAPK and NF- κ B signaling in activated HMC-1 human mast cells

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Abstract

Context: Caffeic acid phenethyl ester (CAPE), an active component of honeybee propolis, is known to have antioxidant, anti-inflammatory, and other beneficial medicinal properties. However, the molecular mechanisms underlying its anti-allergic effects in mast cells are unknown.

Objective: The purpose of the present study was to examine whether CAPE modulates the immunoglobulin E (IgE)-mediated local allergic reaction in animals, as well as to elucidate the effects of CAPE on mast cells *in vitro*.

Materials and methods: To investigate the bioactive potential of CAPE (10 or 20 μ M), HMC-1 cells were stimulated with phorbol 12-myristate 13-acetate plus calcium ionophore A23187 (PMACI) for 24 h in the presence or absence of CAPE. To study the pharmacological effects of CAPE, enzyme-linked immunosorbent assays (ELISAs), RT-PCR, Western blot analysis, electrophoretic mobility shift assays (EMSAs), and fluorescence assays were used.

Results: CAPE (10 mg/kg) inhibited local IgE-mediated allergic reactions (0.164 versus 0.065 O.D.) in a mouse model. Additionally, CAPE (20 μ M) attenuated PMACI-stimulated histamine release (3146.42 versus 2564.83 pg/ml) and the production of inflammatory cytokines, such as interleukin (IL)-1 β (4.775 versus 0.713 pg/ml, IC₅₀ = 6.67 μ M), IL-6 (4771.5 versus 449.1 pg/ml, IC₅₀ = 5.25 μ M), and IL-8 (5991.7 versus 2213.1 pg/ml, IC₅₀ = 9.95 μ M) in HMC-1 cells. In activated HMC-1 cells, pretreatment with CAPE decreased the phosphorylation of c-Jun N-terminal kinase. In addition, CAPE inhibited PMACI-induced nuclear factor (NF)- κ B activation by suppressing I κ B α phosphorylation and its degradation.

Discussion and conclusion: Our results indicated that CAPE can modulate mast cell-mediated allergic disease.

Keywords

Allergy, cytokine, histamine, immunoglobulin E, passive cutaneous anaphylaxis

History

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Introduction

Caffeic acid phenethyl ester (CAPE, empirical formula C₁₇H₁₆O₄), a biologically active component of honeybee propolis, has a structure similar to flavonoids, with a

molecular weight of 284.31 g mol⁻¹ (Figure 1). CAPE has been used in folk medicine since ancient times and has numerous potentially beneficial properties, including anti-viral, antitumoral, anti-inflammatory, antioxidant, neuroprotective, antiatherosclerotic, and immunomodulatory effects in diverse systems (Fesen et al., 1993; Grémy et al., 2006; Hepsen et al., 1999; Hishikawa et al., 2005; Ilhan et al., 2004; Liao et al., 2003; Orban et al., 2000; Park & Kang, 1999; Park et al., 2004). The varied effects of CAPE might be attributed to its ability to traverse the cell membrane (Orsolić et al., 2005). CAPE inhibits the activities of certain enzymes including lipoxygenase, cyclooxygenase, glutathione S-transferase, and xanthine oxidase (Koshihara et al., 1984;

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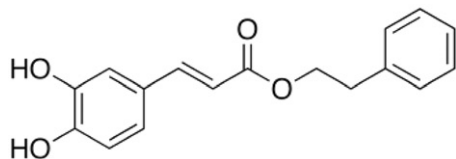


Figure 1. The chemical structure of caffeic acid phenethyl ester (CAPE).

Michaluart et al., 1999); it is also a potent and specific inhibitor of nuclear transcription factor- κ B (NF- κ B) activation (Márquez et al., 2004; Natarajan et al., 1996; Orban et al., 2000).

Allergic disorders, such as bronchial asthma, atopic dermatitis, allergic rhinitis, and food allergies, are typified by undesirable reaction to harmless elements in the environment. Allergic diseases are characterized by the infiltration and the accumulation of lymphocytes, basophils, eosinophils, and mast cells at site of inflammation. Among these cells, mast cells play an important role in promoting allergic response. Mast cells also play an important role in the immune response to many pathogens, including parasites (Metz & Maurer, 2007). Mast cells are commonly found at sites exposed to the external environment, namely the skin, the airways, and the gastrointestinal tract (Galli et al., 1999; Marshall, 2004). Typically, mast cells are considered associated not only with immediate-type hypersensitivity but also with late reactions such as inflammatory responses, which are mast cell dependent (Kemp & Lockey, 2002; Metzger et al., 1986). Mast cells, similar to basophils, constitutively express substantial numbers of Fc ϵ RI, the high-affinity receptor for IgE, on their surface; the number of surface Fc ϵ RI molecules is positively regulated by ambient concentrations of IgE (Kawakami & Galli, 2002). Ag- and IgE-dependent activation of mast cells initiates a complex secretory response by way of aggregation of Fc ϵ RI, when the cells' surface-bound-Fc ϵ RI IgE recognizes bi- or multi-valent Ag. Once activated, mast cells release chemotactic and inflammatory cytokines, such as tumor necrosis factor (TNF)- α , transforming growth factor (TGF)- β , interleukins (IL)-1 β , IL-6, IL-8, IL-13, and immunoregulatory mediators including histamine, serotonin, arachidonic derivatives, and proteases, in a coordinated network (Royer et al., 2001; Stassen et al., 2001; Zhu et al., 1999). The release of these various cytokines can initiate the immediate hypersensitivity responses associated with allergies. Because mast cell numbers are amplified at sites of inflammation in allergic disease, pharmacologic intervention of the proliferation, migration and survival of mast cells could be a promising strategy for the management of allergic diseases.

In the present study, we investigated the effect of CAPE on mast cell-mediated allergic inflammation and studied the possible mechanism(s) of action. The spectrum of inflammatory mediators produced by human mast cell line, HMC-1 cells, on stimulation with PMA plus A23187 (PMACI) supports the well-recognized role of mast cells in immediate hypersensitivity. We therefore evaluated the anti-allergic effects of CAPE on the IgE-dependent passive cutaneous anaphylaxis (PCA) reaction *in vivo*.

Materials and methods

Animals

Male (6–8 weeks old) ICR mice were purchased from Orient Biotech Inc. (Seoul, Korea) and allowed to acclimatize to our animal facility for at least 1 week. All the experimental animals used in this study were maintained under a protocol approved by the Institutional Animal Care and Use Committee of Inje University Medical School.

Materials

CAPE, phorbol 12 myristate 13-acetate (PMA), calcium ionophore A23187 (Calcymycin; C₂₉H₃₇N₃O₆), anti-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA), and Iscove's modified Dulbecco's medium (IMDM) were purchased from the Sigma Chemical Co. (St. Louis, MO). The NF- κ B antibody was obtained from eBioscience (San Diego, CA). Antibodies against JNK, phospho (p)-JNK, p-ERK 1/2, p-p38, and p-I κ B α were purchased from Cell Signaling (Danvers, MA). Antibodies against ERK, p38, and I κ B α were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Cell culture

HMC-1 cells, a human mast cell line, were provided by D.-K. Kim (Chonbuk National University Medical School, Jeonju, Korea). The HMC-1 cells were grown in IMDM and supplemented with 100 units/ml of penicillin, 100 μ g/ml of streptomycin, and 10% fetal bovine serum (FBS) at 37 °C in 5% CO₂ with 95% humidity. HMC-1 cells were treated with CAPE (10–40 μ M) for 30 min. The cells were then stimulated with 50 nM of PMA plus 1 μ M of A23187 and incubated at 37 °C for the indicated time periods.

Determination of cell viability

Cellular viability was assessed by the Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan) assay method. Briefly, wells containing 2×10^4 cells/well were treated with CAPE. After incubation for 24 h, the cells were washed twice with PBS. CCK-8 was added to each well and incubated at 37 °C for 1 h, followed by analysis at 450 nm using a microplate reader (Model EL800, BIO-TEK, Winooski, VT).

PCA reaction

The mice were injected intradermally with 500 ng of anti-DNP IgE into each of the three dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. Forty-eight hours later, each mouse received an injection of 100 μ g of DNP-HSA in PBS containing 4% Evans blue via the tail vein. One hour before this injection, CAPE was administered intraperitoneally. Thirty minutes after the challenge, the mice were sacrificed and the dorsal skin was removed for the measurement of the pigment area. The amount of dye was then determined colorimetrically after extraction with 1 ml of 0.1 N KOH and 9 ml of a mixture of acetone and phosphoric acid (5:13). The absorption intensity of the extraction was measured at 620 nm.

by a spectrometer (SpectraMax M2, Molecular Devices, Sunnyvale, CA).

Histamine assay

The HMC-1 cells were pretreated with various concentrations of CAPE (10 or 20 μ M) for 30 min before PMACI stimulation. The amount of histamine was assayed using an ELISA kit (Oxford Biomedical Research, Rochester Hills, MI), in accordance with the manufacturer's instructions.

Intracellular calcium measurement

To determine intracellular calcium levels, the HMC-1 cells (5×10^6 cells/ml) were pre-incubated with 5 μ M of Fluo-4/AM for 1 h at 37 °C and then harvested. After two washes with calcium-free medium (media containing 3 mM EGTA), the cells were incubated for 10 min at 37 °C and then placed in a 96-well plate and pretreated with CAPE for 10 min before adding PMACI. The intracellular calcium levels were measured at 180 s following treatment using a fluorescent plate reader (SpectraMax M2, Molecular Devices, Sunnyvale, CA) at an excitation wavelength of 494 nm and an emission wavelength of 516 nm.

Cytokine assay

The HMC-1 cells were pretreated with various concentrations of CAPE (10 or 20 μ M) for 30 min before PMACI stimulation. The levels of IL-1 β , IL-6, IL-8, and TNF- α were measured using ELISA kits (BioLegend, San Diego, CA). The quantification was performed using an ELISA plate reader (Dynatech MR-7000; Dynatech Laboratories, El Paso, TX) set to a wavelength of 450 nm, according to the manufacturer's instructions.

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Total RNA was isolated using the TRIzol reagent (Invitrogen, Carlsbad, CA). The total RNA (1.0 μ g) from the cells was reverse-transcribed using M-MLV reverse transcriptase (Promega, Madison, WI) to produce cDNA. RT-generated cDNAs encoding IL-1 β , IL-6, IL-8, and TNF- α were amplified by PCR using selective primers (Table 1). Following amplification, portions of the PCR reactions were subjected electrophoresis on an agarose gel.

Western blot analysis

The cells were washed three times with PBS and lysed with lysis buffer (Mammalian Cell-PE LB, G Biosciences, St. Louis, MO). Equal amounts of protein were separated on 10% SDS-polyacrylamide minigels and transferred to

nitrocellulose membranes. After incubation with the appropriate primary antibody, the membranes were incubated for 1 h at room temperature with a secondary antibody conjugated to horseradish peroxidase. Following three washes in Tris-buffered saline Tween-20 (TBST), the immunoreactive bands were visualized using the ECL detection system.

Electrophoretic mobility shift assay

Nuclear extracts were prepared using NE-PER nuclear extraction reagent (Pierce, Rockford, IL). As a probe for the gel retardation assay, an oligonucleotide harboring the immunoglobulin κ -chain binding site (κ B, 5'-GATCTCAGAGGGGA CTTTCCGAGAGA-3') was synthesized. A non-radioactive method whereby the 3' end of the probe was labeled with biotin was used in these experiments (Pierce). The binding reactions contained 5 μ g of nuclear extract protein, buffer (10 mM Tris, pH 7.5, 50 mM KCl, 5 mM MgCl₂, 1 mM dithiothreitol, 0.05% Nonidet P-40, and 2.5% glycerol), 50 ng of poly (dI-dC), and 20 fM of biotin-labeled DNA. The reactions were incubated for 20 min at room temperature in a final volume of 20 μ l. The competition reactions were conducted by adding a 100-fold excess of cold κ B to the reaction mixture. The mixture was then separated by electrophoresis on a 5% polyacrylamide gel in 0.5 $\times$ Tris-borate buffer and transferred to nylon membranes. The biotin-labeled DNA was detected using a LightShift chemiluminescent electrophoretic mobility shift assay kit (EMSA) (Pierce).

Statistical analysis

The statistical analyses were conducted using Student's *t* test. The results are represented as the means $\pm$ SD of at least three separate experiments. *p* < 0.05 was considered to be statistically significant.

Results

The effects of CAPE on the viability of HMC-1 cells

Initially, we examined the viability of HMC-1 cells on CAPE treatment using the CCK-8 assay. There was no significant cytotoxicity in HMC-1 cells up to the 20 μ M dose, but cell viability was significantly reduced to 40% at the 40 μ M dose of CAPE (Figure 2). Based on these results, a concentration range of 10–20 μ M was chosen for the subsequent experiments.

The effects of CAPE on the IgE-mediated PCA reaction in mice

To assess the anti-allergic effects of CAPE *in vivo*, we used the PCA model. A local extravasation was induced with a local injection of DNP-IgE followed by an antigenic challenge

Table 1. Information on the primers that were used for RT-PCR.

Genes	NCBI no.	Forward (5'–3')	Size (bp)	Reverse (5'–3')
IL-1 β	NT_022135	TGTCCTGCGTGTGAAAGATGA	391	CAGGCAGTTGGGCATTGGTG
IL-6	NT_007819	GATGGCTGAAAAAGATGGATGC	229	TGGTTGGGTCAGGGGTGGTT
IL-8	NT_022778	ACACTGCGCCAACACAGAAATTA	185	TTTGCTTGAAGTTTCACTGGCATC
TNF- α	NT_113891	CCCCAGGGACCTCTCTAATC	241	GGTTTGCTACAACATGGGCTACA
GAPDH	NT_009759	CGTCTAGAAAAACCTGCCAA	117	TGAAGTCAAAGGAGACCACC

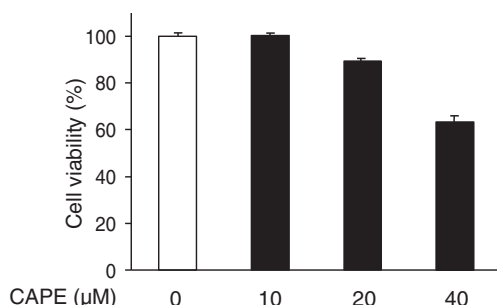


Figure 2. The effect of CAPE on viability in HMC-1 cells. HMC-1 cell (2×10^4 cells) viability was evaluated using the CCK-8 assay at 24 h following treatment with various concentrations of CAPE (10, 20, and 40 μ M). The data are represented as % of the untreated control. All the data are shown as the mean $\pm$ SD from at least three independent experiments performed in triplicate.

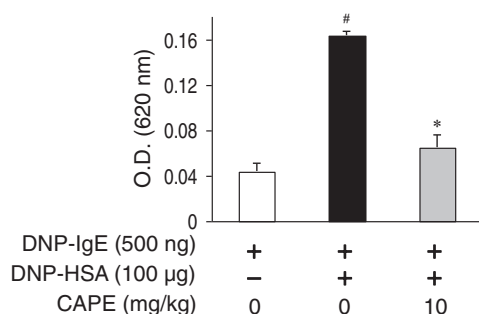


Figure 3. The effect of CAPE on IgE-mediated passive cutaneous anaphylaxis (PCA) in mice. Anti-DNP IgE was injected into dorsal skin sites. CAPE was intraperitoneally administered 1 h before a challenge with 100 μ g of DNP-HSA. The amounts of dye were extracted and measured by spectrometer. All the data are shown as the mean $\pm$ SD from at least three independent experiments performed in triplicate. # p < 0.05, compared with IgE alone values. * p < 0.05, compared with IgE + HSA values.

(DNP-HSA). As shown in Figure 3, the administration of CAPE showed a marked inhibition in the PCA reaction.

The effects of CAPE on histamine release from HMC-1 cells

Among the inflammatory mediators released from mast cells, histamine remains the most well characterized and the most potent vasoactive mediator implicated in the acute phase of hypersensitivity. To investigate whether CAPE inhibits histamine release from mast cells, we measured PMACI-induced histamine release in HMC-1 cells. HMC-1 cells were pretreated with CAPE (10 or 20 μ M) for 30 min before PMACI induction. As shown in Figure 4, the release of histamine in PMACI-treated HMC-1 cells was markedly increased compared to the control group (2276.18 ± 40.73 pg/ml versus 3146.42 ± 177.13 pg/ml). However, pre-treatment with CAPE decreased the release of histamine compared to the PMACI group (2564.83 ± 9.68 pg/ml at the 20 μ M dose).

The effects of CAPE on intracellular calcium levels

To further investigate the mechanism of CAPE on the reduction in histamine release, we assayed the intracellular calcium levels using a spectrometer to detect the fluorescence signal coming from the individual cells (Figure 5). Calcium

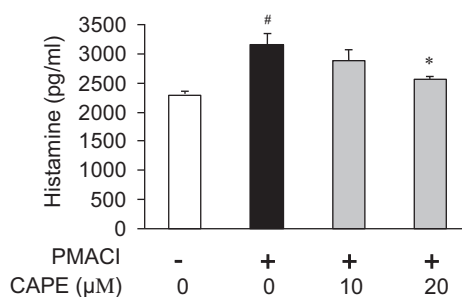


Figure 4. The effect of CAPE on histamine release in PMACI-induced HMC-1 cells. HMC-1 cells (2×10^6 cells/ml) were treated with PMACI for 24 h and the histamine assay was performed on the supernatant from the cells. Each bar represents the mean $\pm$ SD from three independent experiments. # p < 0.05, compared with PMACI-unstimulated cell values. * p < 0.05, compared with PMACI-stimulated values.

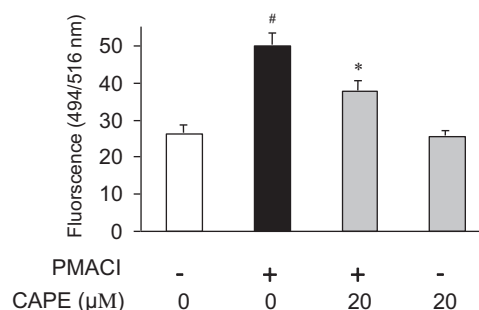


Figure 5. The effect of CAPE on intracellular calcium levels in PMACI-induced HMC-1 cells. The cells were pretreated with CAPE for 10 min before stimulation with PMACI. The intensity of intracellular calcium levels was measured in three separate experiments. # p < 0.05, compared with PMACI-unstimulated cell values. * p < 0.05 compared with PMACI-stimulated values.

uptake into mast cells has been recognized as one of the main events leading to a secretory response; specifically, movement of calcium across the membranes of mast cells results in histamine release. Treatment with CAPE alone resulted in no change in calcium uptake, whereas the PMACI treatment of cells considerably increased the intracellular calcium levels. Pre-treatment of the cells with 20 μ M of CAPE attenuated PMACI-induced calcium uptake.

The effects of CAPE on the expression of pro-inflammatory cytokines in HMC-1 cells

As shown in Figure 6, the levels of IL-1 β , IL-6, IL-8, and TNF- α were considerably increased after stimulation with PMACI in HMC-1 cells. To evaluate the effect of CAPE on the expression of genes encoding pro-inflammatory cytokines, we pretreated cells with CAPE (10 or 20 μ M) before stimulation with PMACI for 3 h. The PMACI-induced expression of IL-1 β , IL-6, and IL-8 in the mast cells was reduced following CAPE treatment. Next, to evaluate the effect of CAPE on the protein expression of pro-inflammatory cytokines, we pretreated cells with CAPE (10 or 20 μ M) before stimulation with PMACI. CAPE treatment suppressed the PMACI-induced protein production of pro-inflammatory cytokines (IL-1 β , IL-6, and IL-8). These results suggested that CAPE inhibits the expression of the cytokines at the transcriptional level. However, these inhibitory effects of CAPE were not found to alter the levels of TNF- α expression.

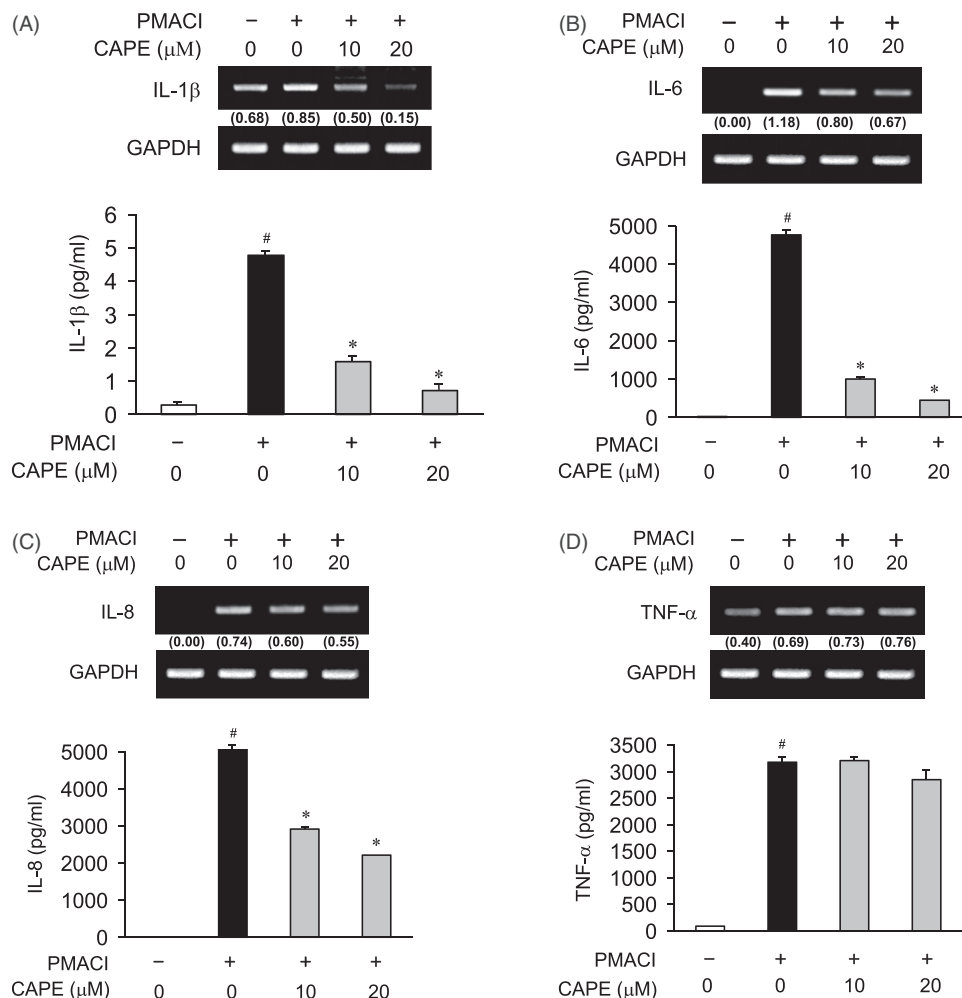


Figure 6. The effect of CAPE on the production of pro-inflammatory cytokines in PMACI-induced HMC-1 cells. The levels of IL-1 β , IL-6, IL-8, and TNF- α mRNA expression were determined by RT-PCR. Cells were pretreated with CAPE (10 or 20 μ M) for 30 min prior to PMACI stimulation for 3 h. The levels of IL-1 β , IL-6, IL-8, and TNF- α protein were determined by ELISA. The cells were pretreated with CAPE (10 or 20 μ M) for 30 min prior to PMACI stimulation for 24 h (IL-1 β , IL-6, and IL-8) and 3 h (TNF- α), respectively. Each bar represents the mean $\pm$ SD from three independent experiments. [#] $p < 0.05$, compared with unstimulated cell values. ^{*} $p < 0.05$, compared with PMACI-stimulated values.

The effects of CAPE on the activation of MAPKs in PMACI-stimulated HMC-1 cells

To elucidate the mechanisms underlying the effects of CAPE, we examined the activation of MAPKs using Western blot analysis. The activation of MAPKs has been shown to induce the production of pro-inflammatory cytokines. The stimulation of HMC-1 cells with PMACI resulted in an increased phosphorylation of all three types of MAPKs: JNK, p38, and ERK. The cells were pretreated for 30 min with CAPE and then treated for 30 min with PMACI. As shown in Figure 7, CAPE attenuated PMACI-induced phosphorylation of JNK, but it did not affect the phosphorylation of ERK and p38 MAPK.

The effects of CAPE on the activation of NF- κ B in PMACI-stimulated HMC-1 cells

The expression of pro-inflammatory cytokines is regulated by the transcription factor NF- κ B. Therefore, to investigate the mechanism by which CAPE affected the expression of pro-inflammatory cytokines, we examined the effects of CAPE on NF- κ B activation. Most inhibitors of NF- κ B activation exert their effects via the suppression of I κ B α phosphorylation and

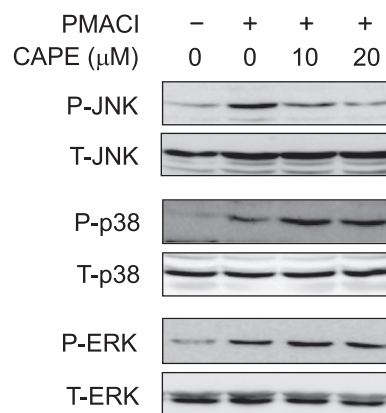


Figure 7. The effect of CAPE on the activation of MAPKs. After pretreatment with CAPE for 30 min, HMC-1 cells were stimulated with PMA (50 nM) + A23187 (1 μ M) for 30 min for MAPK activation. The phosphorylation of MAPKs was analyzed by western blot.

degradation. We found that CAPE inhibited the PMACI-induced phosphorylation and degradation of I κ B α , as well as the nuclear translocation of p65 NF- κ B (Figure 8A). We next investigated the effect of CAPE on the DNA-binding activity of NF- κ B using EMSA (Figure 8B). PMACI treatment caused

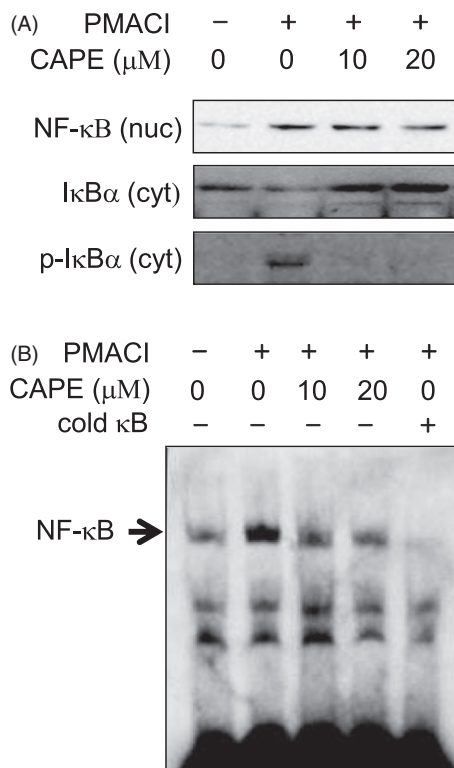


Figure 8. The effect of CAPE on the degradation of IκBα in the cytosol and NF-κB activation in the nuclei of PMACI-stimulated HMC-1 cells. HMC-1 cells were pre-treated with CAPE (10 or 20 μM) for 30 min and then stimulated with PMACI for 30 min. (A) Cytosolic extracts were prepared as described in Materials and methods section and evaluated for IκBα and p-IκBα via Western blot analysis. Nuclear extracts were prepared as described in Materials and methods section and evaluated for NF-κB via Western blot analysis. (B) Nuclear extracts were prepared as described in Materials and methods section and evaluated for NF-κB via EMSA. All the data are presented in the mean ± SD from at least three independent experiments performed in triplicate.

a significant increase in the DNA-binding activity of NF-κB, whereas treatment with CAPE markedly reduced the PMACI-induced DNA-binding activity of NF-κB.

Discussion

Mast cells are important participants in the allergic response. The pleiotropic functions of mast cells reflect their ability to secrete a wide spectrum of preformed or newly synthesized biologically active products with pro-inflammatory, anti-inflammatory, and/or immunosuppressive properties, in response to multiple signals. Human mast cells (HMC-1) are useful cells in the study of immediate-type hypersensitivity because they secrete histamine and many inflammatory cytokines, but only when stimulated with phorbol esters and calcium ionophore A23187 (Kim et al., 2007). Although CAPE has been shown to exhibit anti-allergic effects, its cellular mechanism in mast cells is unknown (Jung et al., 2008; Nader, 2013; Park et al., 2008; Yang, 2011). The purpose of this study was to identify whether CAPE could regulate the signal transduction pathways and the inflammatory mediators, either released or synthesized by the PMACI-induced mast cells.

In this study, we investigated the anti-allergic activity of CAPE on the IgE-mediated PCA in mice. The PCA is a

well-established *in vivo* experimental model of anaphylaxis for evaluating a localized immediate allergic response. When CAPE was administered to mice, it potentially inhibited the PCA response. This finding suggests that CAPE might be a useful candidate in treating allergic diseases.

Calcium is a highly versatile intracellular second messenger that regulates many complicated cellular processes, including cell functions, cell proliferation, and apoptosis (Ma & Beaven, 2009; Suzuki et al., 2010; Yoshimaru et al., 2009). Intracellular calcium is critical to the degranulation of mast cells. The release of preformed granular mediators, such as histamine, serotonin, and β-hexosaminidase, from mast cells is a consequence of complicated biochemical events that occur during the process of degranulation (Ma & Beaven, 2009). Among these granular mediators, histamine has long been known to be a major promoter of allergic inflammatory conditions and gastric acid secretion. It is thus plausible that modulators of intracellular calcium in mast cells might be useful in preventing and relieving the distress of allergic disease. We therefore examined whether CAPE can attenuate intracellular calcium in PMACI-induced HMC-1 cells and found that CAPE indeed reduced the intracellular calcium level in PMACI-treated mast cells. Based on these observations, we speculate that decrease in intracellular calcium may be involved in the inhibitory effect of CAPE on histamine release.

Aggregation of IgE-bound FcεRI by Ag binding induces various inflammatory cytokines that have critical biological roles to play in allergic inflammation. It is essential to understand the signaling pathways and molecules involved in cytokine regulation in mast cells. The reduced level of pro-inflammatory cytokines released from mast cells is a key indicator of reduced allergic responses. In this study, CAPE was found to inhibit the expression of inflammatory cytokines in PMACI-stimulated HMC-1 cells, but did not affect the expression of TNF-α. This result suggests that the anti-allergic inflammatory effect of CAPE may have resulted from its reduction of IL-1β, IL-6, and IL-8, but not TNF-α. TNF-α might not be involved in the regulatory effects of CAPE on the allergy response in our experimental model.

In order to elucidate the inhibitory mechanism of action of CAPE on the expression of inflammatory mediators, we investigated the effect on the activation of MAPKs (ERK, p38, and JNK) in CAPE- and PMACI-treated HMC-1 cells. It has been reported that mast cells induce the expression of cytokines and chemokines via FcεRI-mediated signaling pathways, such as the nuclear factor (NF)-κB and MAPK pathways (Kalesnikoff & Galli, 2008). Our results showed that PMACI simultaneously activated all three MAPKs in HMC-1 cells. However, CAPE inhibited the phosphorylation of JNK but not of p38 MAPK and ERK 1/2 (Figure 7). It is well established that CAPE is a potent and specific inhibitor of NF-κB activation (Natarajan et al., 1996). To further evaluate the mechanism of action of CAPE, we investigated the possible inhibitory effects of CAPE on PMACI-induced NF-κB activation. NF-κB is one of the transcription factors that induce a variety of genes involved in the immune and inflammatory responses. After an inflammatory stimulus, the phosphorylation of IκBα triggers their degradation and the translocation of NF-κB to the nucleus, where it induces

the expression of a broad variety of inflammatory genes, including cytokines, enzymes, adhesion molecules, and acute-phase proteins (Barnes & Karin, 1997). Therefore, NF- κ B is one of the targets that has been identified to treat various diseases. In this study, treating PMACI-induced HMC-1 cells with CAPE attenuated NF- κ B activation and I κ B α degradation. These results suggested that CAPE inhibited pro-inflammatory cytokine production via the inhibition of NF- κ B activation by suppressing I κ B α phosphorylation and its degradation.

Conclusion

In our *in vivo* studies, mice treated with CAPE were protected from IgE-mediated PCA. In addition, CAPE regulated the production of IL-1 β , IL-6, and IL-8 in PMA plus A23187-stimulated HMC-1 cells. CAPE also attenuated histamine and intracellular calcium levels. These inhibitory activities of CAPE occurred via the inhibition of JNK and NF- κ B activation. Taken together, the results obtained in the present study provide evidence that CAPE may be beneficial for the prevention or treatment of mast cell-mediated allergic diseases.

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Declaration of interest

The authors state that they have no conflicts of interest to declare.

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