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Evaluation of the effect of kaempferol in a murine allergic rhinitis model

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ABSTRACT

Kaempferol (KP) is a major compound of Naju Jjok (*Polygonum tinctorium* Lour.). The effect of KP on allergic rhinitis (AR) has not been elucidated. Here, we report the effects and mechanisms of KP on new and predominant mediators of AR using an eosinophil cell line, Eol-1 and an ovalbumin (OVA)-induced AR mouse model. KP significantly inhibited the production of interleukin (IL)-32 and IL-8 and activation of caspase-1 in Eol-1 cells. Allergic symptoms and predominant mediators (IgE and histamine) in the KP-administered group were significantly lower than in the AR group. The levels of interferon-γ were enhanced while the levels of IL-4 were reduced in the KP group. KP significantly reduced the levels of IL-32 and thymic stromal lymphopoietin (TSLP) compared with the AR mice. KP reduced the levels of inflammation-related proteins. In the KP-administered groups, the infiltrations of eosinophils and mast cells increased by OVA were decreased. In addition, KP significantly reduced caspase-1 activity in nasal mucosa tissue of AR mice. Our findings indicate that KP has an anti-allergic effect through the regulation of the production of IL-32 and TSLP and caspase-1 activity in allergic diseases including AR.

1. Introduction

Allergic rhinitis (AR) is a global health problem. Patients, clinicians, and other health care professionals worldwide are faced with the relative merits and downsides of the various treatment options (International Rhinitis Management Working Group, 1994). AR is characterized by the symptoms of sneezing, itchiness, rhinorrhea, and nasal congestion. Patients with AR present an inflammatory IgE-mediated response characterized by a Th2 immunological pattern with mast cells and eosinophil activation and release of inflammatory mediators in response to exposure to allergens (Durham et al., 1992).

Eosinophils are prominent in Th2-driven immune responses, including asthma and allergic and parasitic diseases (Melvin and Ramanathan, 2012). As inflammatory cells, eosinophils contribute to the pathogenesis of allergic inflammation by secreting toxic granule proteins and lipid mediators (Isobe et al., 2012). In addition to their roles in acute inflammation, eosinophils are recognized as immuno-modulatory cells because of interaction with T and B lymphocytes (Bandeira-Melo and Weller, 2005). In particular, granulocyte-macrophage colony-stimulation factor (GM-CSF) is a major survival and activating factor for hematopoietic cells that primes mature macrophages, eosinophils, and neutrophils, and is known as a pleiotropic and pro-inflammatory cytokine (Tai and Spry, 1990). In addition, inflammatory cells in tissues are dependent on adhesion of blood-borne inflammatory cells to endothelial cells before migration to the inflammatory site (Pilewski and Albelda, 1995). Upon stimulation, eosinophils release the tumor necrosis factor (TNF)-α and interleukin (IL)-4, which can modulate adhesion molecules on endothelial cells (Bandeira-Melo and Weller, 2005; Bacon et al., 1998). Activated endothelial cells express the intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 on their cell surfaces (Bacon et al., 1998). Macrophage-inflammatory protein (MIP)-2 is a pivotal chemoattractant for immune cells (Gupta et al., 1996).

IL-32 is a cytokine produced mainly by T lymphocytes, natural killer cells, epithelial cells, mast cells, keratinocytes, eosinophils, and blood monocytes and it has the properties of a classical pro-inflammatory mediator (Kim et al., 2005; Netea et al., 2005). IL-32 induces the overexpression of cyclooxygenase (COX)-2 (Lee et al., 2011). In addition, IL-32 induces the production of proinflammatory cytokines by activation of nuclear factor (NF)-κB and caspase-1 (Kim et al., 2005; Netea et al., 2005; Jeong et al., 2011). Recently, (Jeong et al., 2011) reported that IL-32 released from eosinophils

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significantly increased the levels of IgE and production of inflammatory cytokine, including IL-1β, IL-18, and GM-CSF in AR.

Thymic stromal lymphopoietin (TSLP) has been linked to the pathogenesis of asthma, atopic dermatitis, and AR (Ying et al., 2008; Soumelis et al., 2002; Bunyavanich et al., 2011). TSLP is an important cytokine for the initiation of allergic inflammatory diseases (Song et al., 2012; Allakhverdi et al., 2007). TSLP has been produced through caspase-1 in mast cells (Moon and Kim, 2011). Recently, we reported that IL-32 induced TSLP production by activation of NF-κB and caspase-1 in monocytes (Jeong et al., 2012).

Kaempferol (KP) is a major component of Naju Jjok (*Polygonum tinctorium* Lour.). In this study, we examined the anti-allergic effects of KP on GM-CSF-stimulated Eol-1 cells and ovalbumin (OVA)-induced AR mice.

2. Materials and methods

2.1. Materials

KP, dexamethasone (DEX), OVA, O-phthaldialdehyde (OPA), avidin peroxidase (AP), 2′-azino-bis (3-ethylbenzithiazoline-6-sulfonic acid) tablets substrate (ABTS), purchased 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte-trazolium bromide (MTT), and bicinchoninic acid (BCA) other reagents were purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum (FBS), Roswell Park Memorial Institute (RPMI) 1640, and streptomycin were purchased from Gibco BRL (Grand Island, NY, USA). Anti-mouse IgE/TSLP/IL-4/interferon (IFN)-γ /MIP-2/ICAM-1 antibody (Ab), biotinylated anti-mouse IgE/TSLP/IL-4/IFN-γ/MIP-2/ICAM-1 Ab, recombinant mouse IgE/TSLP/IL-4/IFN-γ/MIP-2/ICAM-1, anti-human IL-8 Ab, biotinylated anti-human IL-8 Ab, and recombinant human IL-8/GM-CSF were purchased from Pharmingen (Sandiego, CA, USA). IL-32 Abs were obtained from BioLegend (San Diego, CA), YbdY Biotech (Seoul, Korea), and Acris (Herford, Germany). Ab for COX-2, caspase-1, and actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Caspase-1 assay kit was supplied by R&D Systems Inc. (Minneapolis, MN, USA).

2.2. OVA-induced AR mouse model

We maintained 6-week-old female BALB/c (Charles River Laboratories, Inc., Wilmington, MA, USA) mice under pathogen-free conditions. Mouse care and experimental procedures were performed under approval from the animal care committee of Kyung Hee University [KHUASP(SE)-11-037]. We sensitized mice on days 1, 5, and 14 by intraperitoneal (i.p.) injection of 100 μg OVA emulsified in 20 mg aluminum hydroxide (Sigma) and then mice were challenged by intranasal (i.n.) injection with 1.5 mg OVA according to previous reports (Choi et al., 2006; Jeong et al., 2011; Pellaton-Longaretti et al., 2011). KP (0.2, 2, or 20 mg/kg) and DEX (5 mg/kg) administrated orally before i.n. OVA challenge for 10 days. The dose of DEX was determined a according to the previous reports (Richter et al., 2003; Masferrer et al., 1990). Nasal symptoms were evaluated by counting the number of nasal rubs that occurred in the 10 min after OVA i.n. provocation at 10 days after challenge. The numbers of mice in each group are 5.

2.3. Culture of Eol-1 cells

Eol-1 cells, human eosinophilic leukemia cell line, are a useful in vitro model for studying human eosinophil functions. The Eol-1 cells have cytological features of myeloblasts under normal culture conditions and differentiate functionally into eosinophils by various stimuli (Mayumi, 1992). Human Eol-1 cells were a kind gift from Dr. Bae H. (Kyung Hee University). The Eol-1 cells were grown in RPMI1640 supplemented with 100 unit/ml penicillin, 100 mg/ml streptomycin and 10% heat-inactivated FBS at 37 °C 5% CO_2 and 95% humidity. Eol-1 cells (3×10^5 cells/ml) were treated with KP (0.2, 2, or 20 μg/ml) for 1 h prior to stimulation with GM-CSF.

2.4. Histamine assay

Histamine of serum was measured by the OPA spectrofluorometric procedure. The fluorescent intensity was measured at 440 nm (excitation at 360 nm) in spectrofluorometer.

2.5. Enzyme-linked immunosorbent assay (ELISA)

Eol-1 cells (3×10^5) were treated with KP (0.2, 2 or 20 μg/ml) for 1 h prior to stimulation with GM-CSF incubated for 24 h. Cytokines of serum, nasal mucosa tissue, and spleen tissue and supernatant were measured by ELISA. ELISA was performed by coating 96-well plates with 1 μg/well of capture Ab. Before the subsequent steps in the assay, the coated plates were washed twice with 1 × PBS containing 0.05% tween-20 (PBST). All reagents and coated wells used in this assay were incubated for 2 h at room temperature. The standard curve was generated from known concentrations of cytokine, as provided by the manufacturer. After exposure to the medium, the assay plates were exposed sequentially to each of the biotin-conjugated secondary antibodies, and AP, and ABTS substrate solution containing 30% H_2O_2. The plates were read at 405 nm. IL-32 was analyzed according to the manufacturers' specifications. Appropriate specificity controls were included, and all samples were run in duplicate. Cytokine level in spleen and nasal mucosa was divided according to the total protein. Protein was determined using a BCA.

2.6. Reverse transcription-polymerase chain reaction (RT-PCR)

Eol-1 cells (3×10^6) were treated with KP (0.2, 2 or 20 μg/ml) for 1 h prior to stimulation with GM-CSF incubated for 4 h. Total RNA was isolated from cells and nasal mucosa according to the manufacturer's specification using easy-BLUETM RNA extraction kit (iNtRON Biotech, Korea). The concentration of total RNA in the final elutes was determined by spectrophotometry. Total RNA (2.5 μg) was heated at 65 °C for 10 min and then chilled on ice. Each sample was reverse-transcribed to cDNA for 90 min at 37 °C using cDNA synthesis kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). PCR was performed with the following primers for mouse TSLP (5′-TAT GAG TGG GAC CAA AAG TAC CG-3′; 5′-GGG ATT GAA GGT TAG GCT CTG G-3′), mouse GAPDH (5′-TTC ACC ACC ATG GAG AAG GC-3′; 5′-GGC ATG GAC TGT GGT CAT GA-3′), human IL-8 (5′-CGA TGT CAG TGC ATA AAG ACA-3′; 5′-TGA ATT CTC AGC CCT CTT CAA AAA-3′), human IL-32 (5′-TGA CAT GAA GAA GCT GAA GGC-3′; 5′-CAT GAC CTT GTC ACA AAA GCT C-3′) and human GAPDH (5′-TCG ACA GTC AGC CGC ATC TTC TTT-3′; 5′-ACC AAA TCC GTT GAC TCC GAC CTT-3′) was used to verify whether equal amounts of RNA were used for reverse transcription and PCR amplification from different experimental conditions. The annealing temperature was 60 °C for TSLP, IL-8, IL-32, and GAPDH. Products were electrophoresed on a 1.5% agarose gel and visualized by staining with ethidium bromide.

2.7. Western blot analysis

Western blot analysis was used nasal mucosa tissue extracts was prepared by detergent lysis procedure. Samples were heated at 95 °C for 5 min, and briefly cooled on ice. Following the centrifugation at 15,000*g* for 5 min, 50 μg aliquots were resolved by 12% SDS-PAGE. Resolved proteins were electrotransferred overnight to nitrocellulose membranes in 25 mM Tris, pH 8.5, 200 mM glycerin, 20% methanol at 25 V. Blots were blocked for at least 2 h with PBST containing