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Inula japonica extract inhibits mast cell-mediated allergic reaction and mast cell activation

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ABSTRACT

Ethnopharmacological relevance: The flowers of *Inula japonica* (Inulae Flos) have long been used in traditional medicine for the treatment of bronchitis, digestive disorders, and inflammation. However, the mechanisms underlying its anti-inflammatory effects remain yet to be elucidated. The objectives of this study were 1) to assess the anti-allergic activity of the ethanol extract of flowers of *Inula japonica* extract (IFE) *in vivo*, 2) to investigate the mechanism of its action on mast cells *in vitro*, and 3) to identify its major phytochemical compositions.

Materials and methods: The anti-allergic activity of IFE was evaluated using mouse bone marrow-derived mast cells (BMMCs) *in vitro* and a passive cutaneous anaphylaxis (PCA) animal model *in vivo*. The effects of IFE on mast cell activation were evaluated in terms of degranulation, eicosanoid generation, Ca^{2+} influx, and immunoblotting of various signaling molecules.

Results: IFE inhibited degranulation and the generation of eicosanoids (PGD_2 and LTC_4) in stem cell factor (SCF)-stimulated BMMCs. Biochemical analysis of the SCF-mediated signaling pathways demonstrated that IFE inhibited the activation of multiple downstream signaling processes including mobilization of intracellular Ca^{2+} and phosphorylation of the mitogen-activated protein kinases (MAPKs), PLCγ1, and $cPLA_2$ pathways. When administered orally, IFE attenuated the mast cell-mediated PCA reaction in IgE-sensitized mice. Its major phytochemical composition included three sesquiterpenes, 1-O-acetylbritannilactone, britanin and tomentosin.

Conclusions: This study suggests that IFE modulates eicosanoids generation and degranulation through the suppression of SCF-mediated signaling pathways that would be beneficial for the prevention of allergic inflammatory diseases. Anti-allergic activity of IFE may be in part attributed particularly to the presence of britanin and tomentosin as major components evidenced by a HPLC analysis.

1. Introduction

Mast cells are major effector cells of allergic inflammation and increasingly recognized for their roles in innate and adaptive immune responses. When mast cells are activated through IgE-dependent or IgE-independent ways, they release preformed mediators from their granules and produce newly synthesized eicosanoids, chemokines and cytokines (Boyce, 2003; Kalesnikoff and Galli, 2008).

Several lines of evidence indicate that various receptors are expressed on the surface of mast cells. Among them, a high-affinity receptor for IgE (FcεRI) is the most well-studied in IgE/antigen-induced responses, whereas the stem cell factor (SCF; also known as Kit ligand) is a cytokine that binds to the c-Kit receptor (CD117), which also induces mast cells development and activation (Roskoski, 2005). Binding of SCF to c-Kit results in dimerization of the receptor followed by activation of its intrinsic tyrosine kinase activity and phosphorylation of key tyrosine residues within the receptor, which leading to a multitude of signaling pathways (Ronnstrand, 2004).

KL and c-Kit binding induces two major signaling pathways by regulating the levels of phosphatidylinositol-4,5-bisphosphate (PIP_2) (Okkenhaug et al., 2007; Rivera and Olivera, 2007).

Abbreviations: IFE, Inulae Flos extract; BMMCs, bone marrow-derived mast cells; IL-3, interleukin-3; LTC_4, leukotriene C_4; SCF, stem cell factor; $cPLA_2$, cytosolic phospholipase A_2; MAPKs, mitogen-activated protein kinases; PLCγ, phospholipase Cγ; AA, arachidonic acid; ERK1/2, extracellular signal-regulated kinase 1/2; JNK, c-jun N-terminal kinases

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One pathway is the phospholipase Cγ (PLCγ)-mediated hydrolysis of PIP_2 to diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3). DAG is a protein kinase C (PKC) activator, while IP_3 binds to specific receptors expressed on the endoplasmic reticulum, triggering the release of Ca^{2+} from internal stores. Ca^{2+} is an important intracellular messenger in mast cells because it plays essential role both mast cell degranulation and arachidonic acid (AA) release and metabolism, which requires the translocation of cytosolic phospholipase A_2 ($cPLA_2$) to intracellular membranes (Evans et al., 2001). The other pathway involves phosphoinositide 3-kinase (PI3K) that phosphorylates PIP_2 to produce PIP_3, which enhances extracellular calcium entry and protein kinase (PKC) activation (Vanhaesebroeck et al., 2001; Wymann and Marone, 2005). Other downstream signal by c-Kit is the Ras/mitogen-activated protein kinases (MAPKs) which have been identified as an important signaling pathway for mitogenic responses and the survival of mast cells (Duronio et al., 1992). The SCF and c-Kit interaction also leads to the activation of many signal transduction pathways. For instance, PI3K regulates the Protein kinase B (Akt), which is a protein-serine/threonine kinase and has been recognized to modulate a wide range of cellular activities (Marone et al., 2008). $cPLA_2$ is a key enzyme that mediates AA metabolism, which is activated by an increase in the intracellular Ca^{2+} concentration and phosphorylation by MAPKs (Clark et al., 1991; Dennis, 1997). The release of AA from cellular membrane phospholipid by $cPLA_2$ interacts with 5-lipoxygenase activating protein (FLAP) that presents AA to 5-lipoxygenase (5-LO) for generating leukotrienes (LTs Murphy and Gijon, 2007).

The flowers of *Inula japonica* Thunb has long been used in traditional Chinese medicine for the treatment of bronchitis, digestive disorders, and inflammation (Liu et al., 2004). However, the underlying mechanisms for its anti-allergic activity have not been elucidated sufficiently. In addition, the effects of IFE on degranulation and the production of eicosanoid that are mediators of inflammatory and allergic reactions also await clarification. In this study, we investigated the anti-allergic activity of IFE on the production of eicosanoids and degranulation in SCF-induced BMMCs, and examined its major phytochemical compositions.

2. Materials and methods

2.1. Plant material

Dried flowers of *Inula japonica* (Inulae Flos) collected from the Anhui province of China were purchased from Ominherb (Youngchun, Korea), and a voucher specimen has been deposited at the Korea Promotion Institute for Traditional Medicine Industry (DGOM-SB09). The Inulae Flos was extracted with ethanol at a ratio of 1:10 (w/v) and then refluxed for 24 h at 60 °C. The extracted solution was filtered, and the solvents were evaporated under vacuum at 40 C (Eyela, Tokyo, Japan), after which they were freeze-dried to obtain the concentrated extract (yield 8%, w/w). The IFE was dissolved in dimethyl sulfoxide (DMSO) and diluted in the medium so that the final concentration of DMSO was less than 0.01% v/v and this concentration of DMSO did not induce mast cell activation. A control of DMSO alone was included in all experiments.

2.2. Reagents

The recombinant mouse SCF was purchased from STEMCELL Technologies Inc (Vancouver, BC, Canada). The primary antibodies used in the experiments were as follows: rabbit polyclonal antibodies specific for phospho-ERK1/2, ERK1/2, phospho-p38, p38, phospho-JNK, JNK, phospho-PLCγ1, and β-actin were from Cell Signaling Technology, Inc. (Danvers, MA, USA); rabbit polyclonal antibodies for phospho-$cPLA_2$ and PLCγ1 were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The horseradish peroxidase-conjugated goat anti-rabbit secondary antibody was also from Cell Signaling Technology, Inc. The LTC_4 and PGD_2 enzyme linked immunoassay (EIA) kit were purchased from Cayman Chemical (Ann Arbor, MI, USA). The enhanced chemiluminescence (ECL) Western blot detection reagent was purchased from Amersham Biosciences, Inc. (Piscataway, NJ, USA).

2.3. Induction of IgE-mediated passive cutaneous anaphylaxis (PCA) reaction in mice

The ICR mice (Hyochang Science, Daegu, Korea) were kept at a temperature of 22 ± 1 °C and at a relative humidity of $55 \pm 10\%$ and a 12 h/12 h (light/dark) cycle for at least 7 d prior to the experiments throughout the study. IFE was dissolved in DMSO and diluted in 0.5% carboxymethyl-cellulose (CMC). Control mice were administrated orally with 0.5% CMC (contain same volume of DMSO) and CMC did not show any cytotoxicity in mice. For PCA, 80 ng of mouse anti-dinitrophenyl (DNP) IgE (Sigma-Aldrich, St. Louis, MO, USA) was intradermally injected into one ear of 7-week-old male mice, followed 24 h later by oral administration of 100–400 mg/kg IFE or 50 mg/kg fexofenadine-HCl, a histanime H1 receptor antagonist (Korea Pharma, Seoul). One hour later, the mice were intravenously challenged with 60 μg of Ag (DNP–human serum albumin (HSA); Sigma-Aldrich, St. Louis, MO, USA) in 200 μl of PBS containing 1% (w/v) Evans blue. The mice were euthanized 1 h after antigen treatment, and their ears were removed and dissolved with 400 μl formamide at 63 °C overnight. The amount of dye extravasation was determined colorimetrically at 630 nm. Experiments using mice were approved by the Institutional Animal Care and Use Committee of Yeungnam University.

2.4. Preparation and activation of BMMCs

Bone marrow cells from male Balb/cJ mice (Sam Taco, INC, Seoul, Korea) were cultured for up to 10 weeks in 50% enriched medium (RPMI 1640 containing 2 mM L–glutamine, 0.1 mM nonessential amino acids, antibiotics and 10% fetal calf serum) and 20% pokeweed mitogen-stimulated spleen condition medium (PWM-SCM) as a source of interleukin-3 (IL-3). After 3 weeks, $>98\%$ of the cells were found to be BMMCs checked by the previously described procedure (Murakami et al., 1994).

2.5. Determination of LTC_4

BMMCs suspended at a cell density of 1×10^6 cells/ml were seeded in a 96-well plate, pre-incubated with various concentrations of IFE for 1 h, and then stimulated with SCF (30 ng/ml) for 15 min. All reactions were stopped by centrifugation at 120g at 4 °C for 5 min, and the supernatants were immediately utilized for LTC_4 determination. The level of LTC_4 was determined using the enzyme immnoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instruction. In these assay conditions, 5-LO dependent LTC_4 generation reached approximately 12 ng/10^6 cells. All data were the arithmetic mean of triplicate determinations.

2.6. Determination of PGD_2

To assess COX-2-dependent PGD_2 generation, BMMCs were preincubated with aspirin (1 μg/ml) for 2 h to irreversibly inactivate the pre-existing COX-1. After washing, BMMCs were activated