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15,16-Dihydrotanshinone I suppresses IgE-Ag stimulated mouse bone marrow-derived mast cell activation by inhibiting Syk kinase

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ABSTRACT

Ethnopharmacological relevance: 15,16-Dihydrotanshinone I (DHT-I), isolated from the dried root of *Salvia miltiorrhiza* Bung, which is traditionally used to treat cardiovascular and inflammatory diseases agent in Chinese medicine. DHT-I has been reported to have a broad range of biological activities, including antibacterial activity, and has been used to treat circulatory disorders, hepatitis, inflammation, cancer, and neurodegenerative diseases.

Aim of the study: The aim of this study was to evaluate the anti-allergic inflammatory effects of DHT-I on degranulation and on the generation of eicosanoids, such as, prostaglandin D_2 (PGD_2) and leukotriene C_4 (LTC_4), in IgE/Ag-stimulated bone marrow-derived mast cells (BMMCs).

Materials and methods: The anti-allergic inflammatory activity of DHT-I was evaluated using BMMCs. The effects of DHT-I on mast cell activation were investigated by following degranulation and eicosanoid generation using ELISA and immunoblotting and immunoprecipitation techniques.

Results: DHT-I at a concentration of 20 μM markedly inhibited degranulation and the generation of PGD_2 and LTC_4 in IgE/Ag-stimulated BMMCs (about 90% inhibitions, respectively). Analyses of FcεRI-mediated signaling pathways demonstrated that DHT-I inhibited the phosphorylations of spleen tyrosine kinase (Syk) and linker for activation of T cells (LAT), and inhibited downstream signaling process, including $[Ca^{2+}]_i$ mobilization induced by the phosphorylation of phospholipase Cγ1 (PLCγ1), and the activations of mitogen-activated protein kinases (MAPKs) and the Akt-nuclear factor-κB (NF-κB) pathway.

Conclusions: DHT-1 inhibits the release of allergic inflammatory mediators from IgE/Ag-stimulated mast cells by suppressing a FcεRI-mediated Syk-dependent signal pathway. This result suggests DHT-I offers a novel developmental basis for drugs targeting allergic inflammatory diseases.

1. Introduction

Salvia miltiorrhiza Bunge (*S. miltiorrhiza*), is a perennial member of the lamiaceae, which are known to contain tanshinones as main constituents and are widely used in traditional Chinese medicine. *S. miltiorrhiza* has many therapeutic uses to treatment of cardiovascular diseases, hepatitis, inflammation, and cancer (Zhou et al., 2005; Wang, 2010). 15, 16-Dihydrotanshinone I (DHT-I) is a constituent of *S. miltiorrhiza,* and has been reported to have a broad range of biological activities, such as, anti-platelet aggregation (Park et al., 2008), anti-inflammatory (Choi et al., 2004; Lee et al., 2006), anti-tumor (Tsai et al., 2007), and antibacterial activities against a broad range of Gram positive bacteria (Lee et al., 1999).

Abbreviations: AA, arachidonic acid; BMMCs, bone marrow-derived mast cells; COX-1,-2, cyclooxygenase-1, -2; $cPLA_2$, cytosolic phospholipase A_2; DMSO, dimethyl sulfoxide; DNP, dinitrophenol; DTT, dithiothreitol; EIA, enzyme immunoassay; ERK1/2, extracellular signal regulated kinase1/2; FBS, fetal bovine serum; Gab2, Grb2-associated binder 2; HSA, human serum albumin; β-Hex, β-hexosaminidase; IKK, IκB kinase; JNK, c-Jun N-terminal kinase; LAT, linker for activation of T cells; LTC_4, leukotriene C_4; 5-LO, 5-lipoxygenase; MAPK, mitogen-activated protein kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide; NF-κB, nuclear factor-κB; PI3K, phosphatidylinositol 3-kinase; PGD_2, prostaglandin D_2; PLCγ1, phospholipase Cγ1; PMSF, phenylmethanesulfonylfluoride; SLP-76, SH2 domain-containing leukocyte protein of 76 kDa; Syk, spleen tyrosine kinase

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Mast cells are granulated cells that play a central role in inflammatory and allergic reactions. The crosslinking of IgE bound to its high-affinity receptor, FcεRI, on mast cells by antigen leads to release of potent inflammatory mediators, such as, histamine, proteases, and *de novo* synthesized lipid mediators, such as, prostaglandin D_2 ($PG)D_2$, leukotriene C_4 $(LT)_4$, platelet-activating factor, and various cytokines and chemokines (Murakami et al., 1995; Yamaguchi et al., 1999). The allergen-induced aggregation of FcεRI on mast cells initiates the activations of tyrosine kinases, such as, Syk, Lyn, Fyn, and BTK, and the phosphorylations of various adaptor molecules (Siraganian, 2003; Gilfillan and Rivera, 2009; Kambayashi and Koretzky, 2007). Syk is essential for the activation of downstream signal molecules, such as, phospholipase Cγ1 (PLCγ1), linker for activation of T cells (LAT), SH2 domain–containing leukocyte protein of 76 kDa (SLP-76), Grb2-associated binder 2 (Gab2), and phosphoinositide 3-kinase (PI3K), which are all essential for intracellular calcium mobilization and degranulation (Siraganian et al., 2010). Previously, we and others groups have suggested the inactivation of Syk kinase could suppress IgE/Ag-stimulated degranulation and the synthesis of eicosanoids and pro-inflammatory cytokines (Rossi et al., 2006; Lu et al., 2011, 2012; Li et al., 2014; Lu et al., 2014).

FcεRI signaling also triggers the activation of mitogen-activated protein kinases (MAPKs), such as, extracellular signal-regulated kinases (ERK1/2), c-Jun N-terminal kinases (JNKs) and p38, PI3K/Akt, and NF-κB signaling pathways, which eventually contribute to the release of various granule-derived mediators, such as, histamine, serotonin, and serine proteases, and induce the expressions of several pro-inflammatory genes, such as, COX-2 and proinflammatory cytokines, which are required for the propagation of inflammation (Tak and Firestein, 2000; Lawrence et al., 2001). In addition, MAPKs play crucial roles in the activation of cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$), which is essential for the release of arachidonic acid from membrane phospholipid, a common precursor of PGD_2 and LTC_4 (Lin et al., 1993; Lu et al., 2011, 2012; Li et al., 2014; Lu et al., 2014). However, the effect of DHT-I on the IgE/Ag-stimulated mast cells has not been studied.

In this study, we examined the effect of DHT-I on mast cell mediator release after IgE-Ag activation in bone marrow-derived mast cells (BMMCs).

2. Materials and Methods

2.1. Chemicals

Mouse anti-dinitrophenyl (DNP) IgE and DNP–human serum albumin (HSA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The rabbit polyclonal antibodies specific for phospho-IκBα, IKKα/β, ERK1/2, JNK, p38, Akt, β-actin and total form for IκBα, ERK1/2, JNK, p38, and Akt, and 5-LO were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Rabbit polyclonal antibodies for phospho-$cPLA_2$ (Ser505), $cPLA_2$, 5-LO, PLCγ1, IKKα/β, lamin B, NF-κB p65, secondary goat anti-rabbit IgG-HRP, rabbit anti-goat IgG-HRP antibodies, total Syk and LAT, and Bay 61–3606 were purchased from Santa Cruz Biotechnology (Dallas, Texas, USA). Antibody for phosphotyrosine was purchased from Millipore (Millipore, Billerica, MA, USA). The antibody-reactive bands were visualized with an enhanced chemiluminescence (ECL) system (Pierce Biotechnology, Rockford, IL, USA). The enzyme immunoassay (EIA) kits for PGD_2, LTC_4 and the antibody for COX-2 were purchased from Cayman Chemicals (Ann Arbor, MI, USA). All other reagents were of the highest analytical grade commercially available.

2.2. Plant material

DHT-I (Fig. 1A) was isolated from the roots of *S. miltiorrhiza* Bunge (Lamiaceae) and structure of DHT-I was verified by comparing NMR data with those reported in the literature (Ryu et al., 1997). DHT-I was prepared by dissolving it in dimethyl sulfoxide (DMSO) diluted with RPMI 1640 medium. The final concentration of DMSO in culture media was adjusted to 0.1% (v/v). DMSO alone was run as a control in all cases. Control experiments showed that DMSO at this concentration had no effect on mast cell activation.

2.3. Culture and activation of bone marrow derived mast cells (BMMCs)

BMMCs were isolated from bone marrow of Balb/cJ mice (Sam Taco, INC, Seoul) and differentiated as described previously (Lu et al., 2011). Briefly, BMMCs were cultured in RPMI 1640 medium (Thermo Scientific, Utah, USA) containing 10% fetal bovine serum (FBS, Thermo Scientific, Utah, USA), 100 U/ml penicillin, 10 mM HEPES, 100 μM MEM non-essential amino acid solution (Invitrogen, Grand Island, NY) and 20% pokeweed mitogen-spleen cell conditioned medium as a source of IL-3. For stimulation, 10^6 cells/ml were sensitized overnight with 500 ng/ml anti-DNP IgE (Sigma), pretreated with indicated concentration of DHT-I or Bay 61-3606 for 1 h at 37 °C, and stimulated for appropriate periods with 100 ng/ml DNP–human serum albumin (HSA; Sigma). The reactions were terminated by centrifugation of the cells at 1000 × g for 5 min at 4 °C.

2.4. Cell viability

Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) assay. Briefly, BMMCs were seeded onto 96 well culture plates at 2×10^4 cells/200 μl/well. After incubation with various concentrations of DHT-I for 8 h, 20 μl of MTT (5 mg/ml) was added to each well. After 4 h incubation, 150 μl of culture medium was removed, and cells were dissolved in 0.4 N HCl/isopropyl alcohol. The optical densities (OD) at 570 nm and 630 nm were measured using a microplate reader (Sunrise, Tecan, Switzerland).

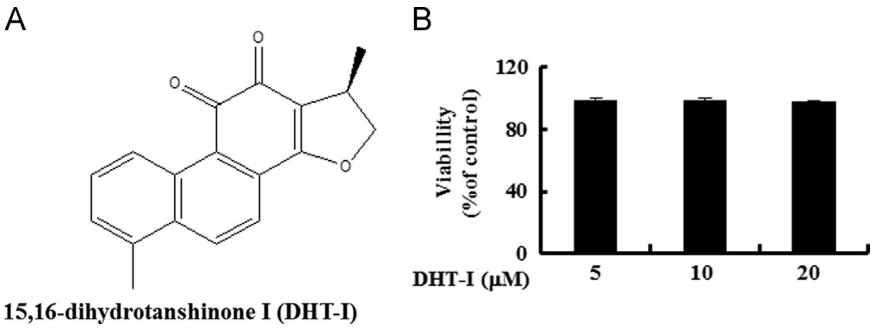

Fig. 1. Chemical structures of 15,16-dihydrotanshinone I (DHT-I).