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Cordycepin diminishes thymic stromal lymphopoietin-induced interleukin-13 production

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ABSTRACT

Atopic dermatitis (AD) is known to aggravate by thymic stromal lymphopoietin (TSLP) and TSLP is also known to up-regulate mast cell proliferation via production of interleukin (IL)-13. Thus, we investigated whether cordycepin could regulate mast cell proliferation induced by TSLP in human mast cell line, HMC-1 cell. Cordycepin significantly diminished the production and mRNA of IL-13 through the down-regulation of phosphorylated-signal transducer and activation of transcription 6 in the TSLP-stimulated HMC-1 cells. Cordycepin also significantly diminished the cell proliferation via down-regulating MDM2 and Bcl2 levels and up-regulating p53, caspase-3, and cleaved poly ADP-ribose polymerase levels in the TSLP-stimulated HMC-1 cells. Moreover, cordycepin significantly diminished the production of IL-6, tumor necrosis factor-α, and IL-1β in the TSLP-stimulated HMC-1 cells. In conclusion, our study shows that cordycepin has potential effect for the treatment of allergic inflammatory diseases through the blockade of IL-13 and MDM2 exacerbated by TSLP.

1. Introduction

Atopic dermatitis (AD) is a common chronic, itchy, highly pruritic, and inflammatory skin disorder that develop asthma, hay fever, or allergic rhinitis. It is a serious skin condition with significant social and financial burden to the patient, their families, and society overall (Leung, 2000). Mast cells act a key player in AD via multiple mechanisms (Rivera and Gilfillan, 2006). Mast cell activation induced by IgE receptor (FcεRI) is considered to be an important event in the allergic inflammatory reactions via secretion of preformed mediators and newly generated mediators, including histamine, prostaglandin D2, proteoglycans, platelet-activating factor, leukotriene C4, tryptase, chymase, and inflammatory cytokines such as thymic stromal lymphopoietin (TSLP) (Gilfillan and Tkaczyk, 2006; Schwartz et al., 1987; Han et al., 2014).

The high TSLP levels are associated with AD, asthma, and food allergies, and many studies proved that TSLP accelerates TH2 cytokine-mediated immune responses and inflammatory reactions by influencing mast cell, dendritic cell, and lymphocytes (Siracusa et al., 2011). TH2 cytokine, IL-13, promotes mast cell proliferation and is implicated in the development of AD (Kaur et al., 2006). IL-13 signaling pathway is tightly connected to signal transducer and activator of transcription (STAT)6 and conducts a vital role in TH2 polarization of the immune system (Hebenstreit et al., 2006). STAT6 also conducts a key role in TSLP-induced mast cell proliferation and survival through the activation of murine double minute (MDM) 2, which is overexpressed and amplified in many human malignancies (Han et al., 2014; Jones et al., 1998; Yoou et al., 2016). The MDM2 is an E3 ligase and regulates degradation of p53 (Honda et al., 1997).

Cordyceps militaris is a potential source of herbal drugs and synthesize cordycepin (3′-deoxyadenosine). Cordycepin has various pharmacological effects including anti-proliferative, immunological stimulating, anti-virus, anti-cancer, anti-infection, and anti-inflammatory activities (Kim et al., 2006; Wong et al., 2010). However, the effects of cordycepin in TSLP-induced inflammatory reaction have not yet been examined. Herein, we evaluated the anti-allergic inflammatory activity of cordycepin in TSLP-stimulated human mast cell line, HMC-1 cells.

2. Material and methods

2.1. Reagents

We bought fetal bovine serum (FBS) and Isocove's modified

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Dulbecco's medium (IMDM) from Gibco BRL (Grand Island, NY, USA); Recombinant TSLP, caspase-3 assay kit, IL-13, tumor necrosis factor (TNF)-α, IL-6, and IL-1β antibodies from R & D Systems, Inc. (Minneapolis, MN, USA); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and anti-phospho-STAT6 (pSTAT6) from Sigma Chemical Co (St. Louis, MO, USA); Bcl2 (Cat. No. SC-7382), procaspase-3 (Cat. No. SC-7148), Poly-ADP-ribose polymerase (PARP, Cat. No. SC-7150), MDM2 (Cat. No. SC-965), and actin (Cat. No. SC-8432) from Santa Cruz Biotechnology (Dallas, TX, USA); bromodeoxyuridine (BrdU) from Roche Diagnostics (Mannheim, Germany). Cordycepin was isolated from *Cordyceps miltaris* (Yoou et al., 2016).

2.2. HMC-1 cells culture

The HMC-1 cells (HMC-1.2) were kindly provided by Eichi Morri (Osaka University, Japan) and HMC-1.2 has two KIT mutations (codon V560G and codon D816V). HMC-1 cells were cultured in Isocove's Modified Dulbecco's Medium with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in 5% CO_2 with 95% humidity.

2.3. Cytokines assay

The levels of IL-13, TNF-α, IL-6, and IL-1β were determined using a sandwich ELISA method according to the manufacturer's instructions (R & D Systems). Briefly, ELISA was performed by coating 96-well plates with 1 μg/well capture antibody. Before the subsequent steps in the assay, the coated plates were washed twice with 1× phosphate-buffered saline with Tween 20 (PBST). All reagents and coated wells used in this assay were incubated for 2 h at room temperature. A standard curve was generated from the known concentrations of cytokine, as provided by the manufacturer. After exposure to the medium, the biotin-conjugated secondary antibody and an avidin peroxidase and ABTS solution containing 30% H_2O_2. The plates were read at 405 nm.

2.4. RNA isolation and quantitative real-Time PCR

Using an easy-BLUE™ RNA extraction kit (iNtRON Biotech, Sungnam, Korea), we isolated the total RNA from HMC-1 cells in accordance with the manufacturer's specifications. The concentration of total RNA in the final elutes was determined by NanoDrop spectrophotometry (Thermo scientific, Worcester, MA, USA). Total RNA (2.5 μg) was heated at 75 °C for 5 min and then chilled on ice. Each sample was reverse-transcribed to cDNA for 60 min at 42 °C using a cDNA synthesis kit (iNtRON Biotech, Sungnam, Korea). Quantitative real-Time PCR was performed using a SYBR Green master mix and the detection of mRNA was analyzed using an ABI StepOne real-time PCR System (Applied Biosystems, Foster City, CA, USA). We performed real-time with the following primers: IL-13 (5′ GCCCTGGAATCCCTGATCA 3′; 5′ GCTCAGCAT CCTCTGGGTCTT 3′); GAPDH (5′ TCGACAGTCAGCCGCATCTTCTTT 3′; 5′ ACCAAA TCCGTTGACTCCGACCTT 3′). The level of the target mRNA was normalized to the level of the GAPDH and compared with the control. All data were analyzed using the ΔΔCT method.

2.5. Cell viability assay

To estimate the cell viability, the MTT assay was performed. Briefly, 500 μl of HMC-1 cell (3×10^5) were pretreated with diverse concentrations of cordycepin for 1 h and stimulated with TSLP for 48 h. The cell suspension containing MTT stock solution (5 mg/ml) was incubated at 37 °C for an additional 4 h. After washing the supernatant out, the crystallized formazan products were dissolved in DMSO. Then, the optical density was determined using an ELISA reader at 540 nm.

2.6. Western blot analysis

The activated cells were lysed and separated through 10% SDS-polyacrylamide gel electrophoresis. After electrophoresis, the protein was transferred to nitrocellulose membranes and then the membranes were blocked and incubated with primary (1:500 dilution in PBST) and secondary (1:3000 dilution in PBST) antibodies. Finally, blots were developed by peroxidase-conjugated secondary antibodies, and proteins were visualized by enhanced chemiluminescence procedures (Amersham Bioseciences, Piscataway, NJ, USA) in accordance with the manufacturer's instructions.

2.7. BrdU assay

Cell (1×10^4) proliferation was analyzed with a BrdU assay kit (Roche Diagnostics GmbH, Mannheim, Germany).

2.8. Caspase-3 assay

The enzymatic activity of caspase-3 was analyzed with a colorimetric assay kit (R & D Systems).

2.9. Statistics

All results are representative of three independent experiments with duplicate and expressed as the mean ± SEM. The statistical evaluation of the results was performed by an independent *t*-test and a one-way analysis of variance with a Tukey post hoc test using SPSS statistical software (IBM Corporation, Armonk, NY, USA). A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Regulatory effect of cordycepin on the levels of IL-13 in HMC-1 cells

IL-13 is a key effector cytokine in allergic inflammatory reactions and is secreted by TSLP from mast cells (Junttila et al., 2013). IL-13 also increased mast cell proliferation (Kaur et al., 2006). To estimate the regulatory effect of cordycepin on IL-13 production and mRNA expression, we performed the ELISA and real time-PCR. TSLP significantly increased the production and mRNA expression of IL-13 on HMC-1 cells (Fig. 1A and B, $P < 0.05$). However, cordycepin significantly diminished the TSLP-induced IL-13 production and mRNA expression in a dose-dependent manner. Maximum inhibition rates by cordycepin (10 μM) on IL-13 production and mRNA expression were about 92% and 80%, respectively (Fig. 1A and B, $P < 0.05$). Cytotoxicities were not showed at doses of 0.1, 1, and 10 μM of cordycepin (Fig. 1C).

3.2. Regulatory effect of cordycepin on the levels of pSTAT6 in HMC-1 cells

STAT6 acts as a proliferator or differentiator of mast cells (Suzuki et al., 2000) and is closely related to IL-13 signaling pathway (Hebenstreit et al., 2006). Thus, we determined the regulatory effect of cordycepin on the level of pSTAT6 by TSLP in HMC-1 cells. TSLP increased the levels of pSTAT6 in HMC-1 cells (Fig. 2). However, cordycepin significantly diminished the TSLP-induced pSTAT6 levels (Fig. 2, $P < 0.05$). Cordycepin alone (10 μM) did not affect the STAT6 phosphorylation (Fig. 2).

3.3. Regulatory effect of cordycepin on the levels of MDM2 and p53 in HMC-1 cells

TSLP promotes the mast cell proliferation via the activation of