Cordycepin Suppresses MHC-restricted Antigen Presentation and Leads to Down-regulation of Inflammatory Responses in Antigen Presenting Cells

Seulmee Shin¹, Seulah Kim¹, Bobae Hyun¹, Aerí Lee¹, Sungwon Lee¹, Chan-Su Park², Hyunseok Kong¹, Youngcheon Song¹, Chong-Kil Lee², and Kyungjae Kim¹*¹

¹College of Pharmacy, Sahmyook University, Seoul 139-742, Republic of Korea ²College of Pharmacy, Chungbuk National University, Cheongju 361-763, Republic of Korea

Abstract – Cordyceps militaris, a traditional medicinal mushroom, produces a component compound, cordycepin (3’-deoxyadenosine). Cordycepin has many pharmacological activities including immunological stimulating, anti-cancer, and anti-infection activities. However, the therapeutic mechanism has not yet been elucidated. In this study, we examined the effects of cordycepin on the antigen-presenting function of antigen-presenting cells (APCs). Dendritic cells (DCs) were cultured in the presence of cordycepin and then allowed to phagocytose microspheres containing ovalbumin (OVA). After washing and fixing, the efficacy of OVA peptide presentation by DCs was evaluated using CD8 and CD4 T cells. Also, we confirmed the protein levels of proinflammatory cytokines through RT-PCR and Western blot analysis. Cordycepin decreased both MHC class I and class II-restricted presentation of OVA and suppressed the expression of both MHC molecules and the phagocytic activity toward exogenous OVA. The class II-restricted OVA presentation-regulating activity of cordycepin was also confirmed using mice that had been injected with cordycepin followed by soluble OVA. Furthermore, cordycepin suppressed the mRNA and protein levels of iNOS, COX-2, pro-inflammatory cytokines in a concentration-dependent manner. These results provide an understanding of the mechanism of the T cell response-regulating activity of cordycepin through the inhibition of MHC-restricted antigen presentation in relation to its actions on APCs.

Keywords – Cordycepin, Inflammation, MHC-restricted antigen presentation, Antigen-presenting cells

Introduction

Cordycepin, also known as 3’-deoxyadenosine, is a bioactive compound present in fungi species belonging to the genus Cordyceps (Cunningham et al., 1950) and was first isolated from Cordyceps militaris. C. militaris is commonly used in East Asian countries to maintain health and to prevent and treat a broad spectrum of illnesses including those of the circulatory, immune, respiratory, and glandular systems. Cordycepin has various biological effects including anti-fungal (Sugar and McCaffrey, 1998), anti-malarial (Trigg et al., 1971), anti-herpes (Julian-Ortiz et al., 1999), anti-tumorigenic, anti-leukemic (Deitch and Sawicki, 1979), anti-inflammatory (Shin et al., 2009; Shin et al., 2009), anti-diabetic (Yun et al., 2003; Lo et al., 2004; Kim et al., 2006) and anti-oxidant effects (Ramesh et al., 2012). Cordycepin also induces apoptosis in many cells and induces cell cycle arrest by targeting molecules and pathways (Lee et al., 2009; Lee et al., 2010; Lee et al., 2013). Even though cordycepin exhibits a number of pharmacological properties, further studies are needed to address these pharmacological differences and little known about its immune-regulating effects or its effects on antigen presentation in T cell responses based on the capability of antigen-presenting cells (APCs).

Antigen-presenting cells, especially dendritic cells (DCs), play a critical role in the initiation of immune responses and the induction of immune tolerance. DCs are the most important accessory cells for the activation of naïve T cells and the generation of primary T cell responses (Banchereau et al., 2000). They can acquire and process antigens in the periphery and then migrate to secondary lymphoid tissues where they prime primary T cell responses. The activation of T cells and subsequent generation of the effector function are dependent on MHC molecules, and modulation of MHC-restricted antigen processing pathways may provide novel pharmacological targets for the regulation of T cell responses. In fact, we previously showed that cyclosporin A (CsA) and tacrolimus

*Author for correspondence
Kyungjae Kim, College of Pharmacy, Sahmyook University, Seoul, Republic of Korea
Tel: +82-2-3399-1601; E-mail: kimkj@syu.ac.kr
inhibit both the class I and class II MHC-restricted antigen presentation pathways in DCs (Lee et al., 2005; Lee et al., 2007) and metformin, widely used for type 2 diabetes (T2D) therapy, suppresses MHC-restricted antigen presentation by inhibiting co-stimulatory factors and MHC molecules (Shin et al., 2013). Macrophage colony stimulating factor (M-CSF) also enhances MHC-restricted antigen presentation (Han et al., 2005), and Cordyceps militaris water extract (CME) enhances MHC-restricted antigen presentation via the induced expression of MHC molecules and the production of cytokines (Shin et al., 2010). This suggests that modulation of the antigen-specific signal may be useful for therapeutic regulation of T cell responses.

In the present study, we examined the effects of cordycepin on the ex vivo and in vivo function of APCs, exploring the modulation of T cell responses by cordycepin as an immune-regulating agent. We used OVA as an exogenous antigen in conjunction with cordycepin and then compared the change in cross-presentation of metformin-related DCs to that of a control group, along with the levels of MHC class I and II molecules. Also, we tested the role of cordycepin on the NF-κB-dependent inflammation cascades in lipopolysaccharide (LPS) - stimulated macrophages.

Experimental

Cells and reagents – The T cell hybridomas, CD8 OVA1.3 and DOBW, were kindly provided by Dr. Clifford V. Harding (Case Western Reserve University, Cleveland, OH) (Harding et al., 1991; Harding and Song, 1994). The DC cell line, DC2.4, was obtained from Dana-Farber Cancer Institute, Boston, MA, USA (Shen et al., 1997). Cordycepin was purchased from Sigma (St. Louis, MO, USA). The cell culture media DMEM, antibiotic-penicillin/streptomycin solution and fetal bovine serum (Hyclone, Logan, UT, USA) were used for the cell culture.

Preparation of peritoneal macrophages and cell culture – Peritoneal macrophages were elicited by injecting 1 ml of 3% thioglycollate in sterile water into the mouse peritoneum. After 4 days, the cells in the peritoneum were harvested by peritoneal lavage with ice-cold PBS. The red blood cells in the cell preparation were lysed by treatment with ACK lysis buffer (150 mM NH4Cl, 1 M KHCO3, 0.1 mM Na2EDTA, pH 7.2 – 7.4) for 1 min. Cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with high glucose, L-glutamine, 110 mg/L sodium pyruvate, 10% fetal bovine serum (FBS), and 1% (v/v) penicillin (10,000 U/mL)/streptomycin (10,000 U/mL) (P/S).

Isolation of total RNA and RT-PCR – Total RNA was extracted from peritoneal macrophages using the RNeasy Mini kit (Qiagen, USA) in an RNase-free environment. The reverse transcription of 1 μg RNA was carried out using M-MLV reverse transcriptase (Promega, USA), oligo (dT) 16 primer, dNTP (0.5 μM) and 1 U RNase inhibitor. After incubation at 65°C for 5 min and 37°C for 60 min, M-MLV reverse transcriptase was inactivated by heating at 70°C for 15 min. The polymerase chain reaction (PCR) was performed in 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2 and 2.5 mM dNTPs with 5 units of Taq DNA polymerase and 10 pM of each primer set for i-NOS, IL-1β, IL-6, TNF-α, COX-2, and β-actin. The cDNA was amplified by 35 cycles of denaturing at 94°C for 45 s, annealing at 62°C for 45 s, and extension at 72°C for 1 min. Final extension was performed at 72°C for 5 min. The PCR products were electrophoresed on 1.5% agarose gels and stained with ethidium bromide. The primers used were 5’ AGCTCTCT CCCAGGACCACAC 3’ (forward) and 5’ AGCGTGAATCCTCATGGGC 3’ (reverse) for iNOS, 5’ CAGGATGACACATGACACC 3’ (forward) and 5’ CTCGCGAGACTAACTCACC 3’ (reverse) for IL-1β, 5’ GGAACCTGAGG 3’ (forward) and 5’ GGTGCGTGACACTCACCGGCC 3’ (reverse) for IL-6, 5’ TCTGACTCCAGAACACCAGAGG 3’ (forward) and 5’ CCTGTAGCCCACCGGCTGACTGAGC 3’ (reverse) for TNF-α, 5’ AAGAAGAAAG TTCCATTCTGTCCACCC 3’ (forward) and 5’ TCGGTAGCTGACAACCACGCC 3’ (reverse) for β-actin. The β-actin was used as an internal control.

Western blot analysis – Peritoneal macrophages were washed with phosphate-buffered saline (PBS) and lysed in lysis buffer (1% SDS, 1.0 mM sodium vanadate, 10 mM Tris-Cl buffer, pH 7.4) for 5 min. 20 μg of protein from the cell lysates was applied to 8-12% SDS-polyacrylamide gels and then transferred to nitrocellulose membranes. The membranes were blocked with 5% skim milk in PBST solution for 1 hr. They were then incubated with anti-IL-1β, anti-IL-6, anti-TNF-α, anti-iNOS, or anti-COX-2 monoclonal antibody for 2 h and washed 3 times with PBST. After incubation with alkaline phosphatase-labeled secondary antibody for 2 h, the bands were visualized using a Western Blot Kit with alkaline phosphatase substrate (Vector, Burlingame, CA, USA.)

Generation of DCs from bone marrow cells – Total bone marrow cells obtained from the femurs of Balb/c