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Induction of intercellular adhesion molecule-1 by water-soluble components of *Hericium erinaceum* in human monocytes

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ABSTRACT

Aim of the study: Hericium erinaceum is a medicinal mushroom that has been traditionally used in Asian countries for the treatment of cancers and infectious diseases. Although the immunomodulating activity of *H. erinaceum* is considered to be responsible for its medicinal activity, its action mechanisms are poorly understood. In the present study, we investigated the capability of water-extracted *H. erinaceum* (WEHE) to induce the expression of intercellular adhesion molecule-1 (ICAM-1), which regulates the migration of immune cells.

Materials and methods: THP-1, a human monocytic cell-line, or human peripheral blood mononuclear cells (PBMC) were stimulated with WEHE (0–30 μg/mL) and subsequently analyzed using flow cytometry to examine the surface expression of ICAM-1 protein. Steady-state levels of ICAM-1 mRNA were estimated using real-time reverse transcription-polymerase chain reaction analysis. Electrophoretic mobility shift assay was conducted to examine transcription factors involved in ICAM-1 transcription.

Results: WEHE induced ICAM-1 expression at both protein and mRNA levels in THP-1 cells in a dose- and time-dependent fashion. A similar pattern of ICAM-1 induction was also observed in $CD14^+$ monocytes in human PBMC that were stimulated with WEHE. The ICAM-1 expression on THP-1 cells stimulated with WEHE was suppressed by specific inhibitors for extracellular signal-regulated kinases (ERK) and reactive oxygen species (ROS). Additionally, exposure of THP-1 cells to WEHE increased the DNA binding activities of NF-κB, AP-1, SP-1 and STAT-1 transcription factors, all of which are known to be required for ICAM-1 gene expression.

Conclusions: These results suggest that WEHE induces ICAM-1 expression in human monocytes through ERK- and ROS-dependent signaling pathways, resulting in the subsequent activations of NF-κB, AP-1, SP-1, and STAT-1 transcription factors.

1. Introduction

Medicinal mushrooms have been traditionally used in oriental medicine due to their beneficial effects such as anti-cancer and anti-microbial activities in animals and humans (Liu et al., 2010; Zaidman et al., 2005). *Hericium erinaceum*, a well-known traditional medicinal mushroom, has attracted much attention for its anti-tumor, nematicidal, and anti-microbial properties (Mizuno et al., 1992; Stadler et al., 1994). Composition analysis showed that *H. erinaceum* contains various bioactive constituents such as polysaccharides, lectins, proteins, lipids, hericenone, erinacol, erinacine, and terpenoids (Kawagishi et al., 1994; Kenmoku et al., 2002; Lee et al., 2000). Accumulating data suggest that the immunomodulating activity of *H. erinaceum* is responsible for its medicinal activity. Water-soluble extracts of *H. erinaceum* (WEHE) are capable of inducing IL-1β expression (Son et al., 2006b) and nitric oxide (NO) production (Son et al., 2006a) in murine macrophages. Moreover, WEHE augments the cytolytic activity of natural killer (NK) cells against cancer cells through induction of IL-12 expression in murine splenocytes (Yim et al., 2007). WEHE also induces maturation and activation of human dendritic cells (Kim et al., 2010a),

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which play an essential role in innate immunity and subsequent adaptive immunity.

Transmigration of immune cells is an important process for the appropriate activation of the host immune system. Intercellular adhesion molecule-1 (ICAM-1, CD54) is a member of the immunoglobulin superfamily and interacts with β2 integrin molecules such as LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) to trigger trans-endothelial migration of leukocytes into sites of inflammation (Smith et al., 1989; Staunton et al., 1990). Additionally, ICAM-1 participates in the effector function of leukocytes, contributing to the adhesion of antigen-presenting cells to T lymphocytes for antigen presentation, microbial pathogenesis, wound healing and signal transduction pathways (Hubbard and Rothlein, 2000).

ICAM-1 is expressed constitutively but at low levels on the surfaces of various cell types including leukocytes, endothelial cells, keratinocytes, and epithelial cells (Rothlein et al., 1986). ICAM-1 expression is inducible and is up-regulated by oxidative stress, viral infection and inflammatory cytokines such as TNF-α (Pober et al., 1986a), IL-1β (Dustin et al., 1986) and IFN-γ (Pober et al., 1986b). ICAM-1 expression is mainly regulated at the transcriptional level (Voraberger et al., 1991; Wawryk et al., 1991), although post-transcriptional regulation of ICAM-1 expression has also been reported (Most et al., 1992; Wertheimer et al., 1992). ICAM-1 transcription occurs through generation of reactive oxygen species (ROS) (Jung et al., 2008; Kim et al., 2008) and activation of various protein kinases including mitogen-activated protein (MAP) kinases (p38, ERK and JNK) (Ho et al., 2008; Tamura et al., 1998), protein kinase C (PKC) (Min et al., 2005), and phosphatidylinositol-3-kinase (PI3K) (Radisavljevic et al., 2000), depending on the cell type and stimulant used. These signal transduction cascades activate NF-κB and other transcription factors such as AP-1, SP-1 and STAT-1, leading to maximum induction of ICAM-1 expression (Roebuck and Finnegan, 1999; van de Stolpe and van der Saag, 1996).

Despite the ethnopharmacological importance of *H. erinaceum*, the action mechanisms of its immunomodulating ability are still poorly understood. In the present study, we investigated the inductive effects of WEHE on ICAM-1 expression and its downstream signaling pathways in the human monocytic cell-line, THP-1.

2. Materials and methods

2.1. Reagents and chemicals

Phycoerythrin (PE)-labeled antibody to human ICAM-1 (also called CD54) and allophycocyanin (APC)-labeled antibody to human CD14 were purchased from BD Biosciences (San Diego, CA, USA). PD98059, U0126, SB203580, SB202190, SP600125, N-acetyl cysteine (NAC), LY294002, and calphostin C were purchased from Calbiochem (Darmstadt, Germany). Lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4 was purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Preparation of WEHE

WEHE was prepared from dried *H. erinaceum* and deposited at the herbarium of Daejon University (Voucher specimen number: HE-2005-01-Fr), as previously described (Son et al., 2006a, b; Yim et al., 2007). Briefly, 100 g of the dried mushroom was subjected to intensive washing with pyrogen-free distilled water followed by soaking in 1.5 L of water for 2 h and boiling for 2 h. The water-insoluble components were removed by centrifugation at 3000 × *g* for 30 min, and the aqueous fraction was separated for lyophilization. At the end of this process, about 26 g of lyophilized extract (named as WEHE) was obtained. The composition of WEHE was analyzed using the standard methods recommended by the Association of Official Analytical Chemists, as previously described (Son et al., 2006b; Yim et al., 2007). The WEHE used in the present study was composed of proteins (44.82%), carbohydrates (27.63%), ashes (16.83%), moisture (9.05%), fibers (0.94%), and fats (0.72%).

2.3. Gas chromatography–mass spectrometry (GC–MS) analysis of WEHE

Lyophilized WEHE was derivatized in two steps to protect carbonyl function. First, dried samples were dissolved in 100 μL of 20 mg/mL solution of methoxyamine hydrochloride in pyridine (Sigma–Aldrich) and reacted at 60 °C for 60 min. The acidic protons were exchanged against the trimethylsilyl group in order to increase the volatilities of the polar compounds using 100 μL *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA, Sigma–Aldrich) at 70 °C for 60 min. One microliter of the sample was analyzed using gas chromatography coupled with electron impact and ion trap tandem mass spectrometry (GC-EI-IT-MS) in a CP-3800 gas chromatograph coupled to a 4000 ion trap mass spectrometer equipped with a CP-8400 auto sampler (Varian, CA, USA). The injection temperature was set at 250 °C, and the samples were analyzed using a standard split inlet (split ratio 25:1) equipped with a Varian VF-1 MS capillary column (30 m × 0.25 mm i.d. 0.25 μm film). The flow rate of helium carrier gas (purity > 99.999%) through the column was 1 mL/min. The column temperature was held isothermally at 70 °C for 4 min and then raised at 10 °C/min to 300 °C and maintained isothermally for 6 min. The interface and ion source were adjusted to 200 °C, and electron impact ionization was performed at 70 eV. Scan average was set to 3 μscans, with full scan in the range of 50–1000 *m*/*z*. Metabolites were identified by comparison to the NIST Mass Spectral Search Program 2005 database (version 2.0, FairCom Co., Columbia, MO, USA).

2.4. Culture of THP-1 cells

THP-1, a human monocytic cell-line, was obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were grown in RPMI 1640 medium (Invitrogen, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (HyClone, Logan, UT, USA), 100 U of penicillin per mL, and 100 μg of streptomycin (Invitrogen) per mL at 37 °C in a humidified incubator with 5% CO_2.

2.5. Isolation of peripheral blood mononuclear cells (PBMC)

Human blood was acquired from healthy volunteers, and PBMC were isolated using density gradient centrifugation with Ficoll-Paque Plus (GE Healthcare Bio-science AB, Uppsala, Sweden). All experiments using human blood were conducted under Institutional Review Board approval at Seoul National University (IRB no. S-D20060001)

2.6. Analysis of ICAM-1 expression using flow cytometry

THP-1 cells were seeded at 3×10^5 cells per well in a 48-well tissue culture plate and treated with various concentrations of WEHE for 24 h or with 30 μg/mL of WEHE for the indicated time periods (0–48 h). Cells were treated with inhibitors 1 h prior to WEHE treatment. After incubation, the cells were harvested and washed twice with phosphate-buffered saline (PBS) containing 2% FBS. To measure ICAM-1 expression, monoclonal PE-conjugated anti-ICAM-1 or isotype-matched antibodies (PE-mouse $IgG_1\kappa$) were added. After incubation on ice for 30 min in the dark, the cells were washed three