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Immunopharmacology and inflammation

Zinc oxide nanoparticles, a novel candidate for the treatment of allergic inflammatory diseases

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ABSTRACT

Zinc (Zn) is an essential trace metal for eukaryotes. The roles of Zn in the numerous physiological functions have been elucidated. Bamboo salt contains Zn that was shown to have anti-inflammatory effect and other health benefits. Nanoparticles of various types have found application in the biology, medicine, and physics. Here we synthesized tetrapod-like, zinc oxide nanoparticles (ZO-NP; diameter 200 nm, source of Zn) using a radio frequency thermal plasma system and investigated its effects on mast cell-mediated allergic inflammatory reactions. ZO-NP was found to inhibit the productions and mRNA expressions of inflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α on the phorbol 12-myristate 13-acetate plus A23187 (PMACI)-stimulated human mast cell line, HMC-1 cells. In these stimulated cells, caspase-1 and nuclear factor-κB activations were abolished by ZO-NP, and the expressions of receptor interacting protein2 (RIP2) and IκB kinaseβ (IKKβ) induced by PAMCI were reduced. On the other hand, ZO-NP alone increased the expressions of RIP2 and IKKβ in normal condition. ZO-NP inhibited the phosphorylation of extracellular signal-regulated protein kinase in the PMACI-stimulated HMC-1 cells. Furthermore, ZO-NP significantly inhibited passive cutaneous anaphylaxis activated by anti-dinitrophenyl IgE. These findings indicate that ZO-NP effectively ameliorates mast cell-mediated allergic inflammatory reaction, and suggest that ZO-NP be considered a potential therapeutic for the treatment of mast cell-mediated allergic diseases.

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1. Introduction

Mast cells reside in all mammalian tissues, and play important roles in the pathogenesis of inflammatory and autoimmune diseases (Heger et al., 2014). Actually, mast cells act as effector cells during early stage of allergic reactions, such as, life-threatening anaphylaxis, allergic rhinitis (hay fever), atopic dermatitis (eczema), and allergic asthma, and during later stage inflammatory response (El-Agamy, 2012; Galli and Tsai, 2012). Activation of mast cell induces degranulation and produces cytokines and chemokines (Galli and Tsai, 2012). Cytokines released by mast cells increase the proinflammatory cytokine production by resident cells. The release of histamine stimulated vessel permeabilization and promoted the migration of eosinophils, neutrophils, and macrophages into the inflammatory zone tissue. Thus the activation and degranulation of mast cells intensifies and extends the inflammatory response (Galli and Tsai, 2012).

Receptor interacting protein2 (RIP2) plays an important role in the regulation of immune response and inflammatory processes, and its signaling tightly linked with IκB kinaseβ (IKKβ), mitogen-activated protein kinases (MAPKs), nuclear factor-κB, and caspase-1 signaling (Perkins, 2007; Song et al., 2012). Furthermore, IKKβ activated by RIP2 triggers the phosphorylation and subsequent ubiquitination of inhibitory IκBα protein, the separation of IκBα and NF-κB, and the degradation of IκBα by proteosomes (Perkins, 2007). The NF-κB so liberated is then translocated into the nucleus and binds to specific DNA sequences to trigger the syntheses of pro-inflammatory mediators, such as, interleukin(IL)-1β, IL-6, IL-8, and tumor necrosis factor-α (TNF-α) (Song et al., 2012). NF-κB is activated by MAPK family members, such as, extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK) (Han et al., 2013). In addition, caspase-1 contributes to NF-κB activation via the autocrine action of IL-1β on cell surface receptors (Wu et al., 2009). Caspase-1 is also characterized by its ability to activate the inactive precursors of inflammatory cytokines (Yoo et al., 2002).

Zinc (Zn) is an essential trace metal for eukaryotes. Zn modulates the numerous physiological functions (Jansen et al., 2009; Maremanda et al., 2014). Bamboo salt contains Zn that was shown to have anti-inflammatory effect and other health benefits (Kim et

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al., 2012). Nanotechnology has been applied in biology, medicine, materials science, engineering, electronics, and environmental fields because of the unique properties of nanoparticles, for example, their superparamagnetic behaviors, small sizes, and great surface areas. Zinc oxide (ZnO) is source of Zn. ZnO decreased expressions of inflammatory genes (Hu et al., 2013; Ou et al., 2007). ZnO nanoparticles (ZO-NP) are used in a wide range of products, such as, in the cosmetics, food packaging, and imaging industries, and as antibacterial and antifungal agents (Roy et al., 2013, 2014; Sharma et al., 2012). In the present study, we synthesized ZO-NP using a radio frequency thermal plasma system and investigated their anti-inflammatory effects in phorbol 12-myristate 13-acetate plus A23187 (PMACI)-stimulated human mast cell line, HMC-1 cells.

2. Materials and methods

2.1. Materials

PMA, A23187, dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), anti-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA), and other reagent were purchased from Sigma (St. Louis, MO, USA). Iacove's modified Dulbecco's medium (IMDM), and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). Anti-human IL-1β, IL-6, IL-8, and TNF-α, biotinylated anti-human IL-1β, IL-6, IL-8, and TNF-α, recombinant human (rh) IL-1β, IL-6, IL-8, and TNF-α were purchased from Pharmingen (San Diego, CA, USA). Antibodies (Abs) for IKK-β, RIP2, caspase-1, ERK, phosphorylated (p) ERK, p38, pp38, JNK, pJNK, NF-κB, IκBα, histone, and actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The caspase assay kit was supplied by R&D Systems Inc. (Minneapolis, MN, USA).

2.2. ZO-NP preparation

As raw material, micron-sized powders of ZnO (particle size 1–10 μm) were purchased from Yee Young Cerachem. Ltd. (Seoul, Korea). ZO-NP was obtained by treating raw powders in a commercial radio frequency (RF) thermal plasma system, consisting of a RF power supply, an induction torch (Tekna, PS-100) for generating the plasma, a synthesis reactor for reforming powder in the high thermal plasma, a cyclone for segregating bulky particles, a filtration unit for gathering the nanoparticles produced, and a powder feeder. The ZnO was reconstituted as a nanoparticles at an operating pressure of ~89.6 kPa, a plate power levels of ~140 kV A, and a feeding rate of 5 g/min. Argon and oxygen was used to produce the plasma flame at the following gas flow rates; central gas of 60 (Ar), sheath gas of 100 (Ar) and 100 (O_2), and quenching gas of 7200 (recycled gas).

2.3. Field emission scanning electron microscopy (FE-SEM), field emission transmission electron microscopy (FE-TEM), and X-ray diffraction (XRD)

Particle morphologies of ZnO and ZO-NP were examined by FE-SEM (S4800, Hitachi Ltd., Japan), FE-TEM (JEM-2200FS, JEOL, Japan), and Bio-TEM (H-7650, Hitachi Ltd., Japan). The FE-SEM was operated at acceleration voltages of 10 kV. FE-TEM or Bio-TEM samples were prepared by dispersing the ZO-NP in desired solvent (ethanol or 10% FBS in IMDM) and drying it on a Cu grid. XDR patterns in 2-theta range of 15° to 90° were obtained for both powders (D8Advance, Bruker AXS GmbH., Germany) using a copper Kα radiation source ($\lambda = 1.5406$ Å).

2.4. Cell culture

HMC-1 cells were grown in IMDM supplemented with 100 U/ml penicillin, 100 mg/ml streptomycin, and 10% heat inactivated FBS at 37 °C, 5% CO_2 and 95% humidity. Powdered ZO-NP and ZnO were prepared by dissolving with DMSO. Dilutions of ZO-NP and ZnO were made in 10% FBS in IMDM. Cells were pretreated with various concentrations of ZO-NP (0.01, 0.1, 1, or 10 μg/ml) or ZnO (0.01, 0.1, 1, or 10 μg/ml) for 1 h prior to PMACI stimulation

2.5. Enzyme-linked immunosorbent assay (ELISA)

Secreted IL-1β, IL-6, IL-8, and TNF-α in culture supernatants were measured according to the manufacturer's specification (Pharmingen, San Diego, CA, USA).

2.6. MTT assay

HMC-1 cells (3×10^5 cells/ml) were treated with ZnO or ZO-NP, cultured in microplate wells for 8 h, and then incubated with 20 μl of MTT solution (5 mg/ml) for an additional 4 h at 37 °C under 5% CO_2 and 95% air. Consecutively, 250 μl of DMSO was added to extract the MTT formazan and the absorbance of each well at 540 nm was read by an automatic microplate reader.

2.7. RNA isolation and RT-PCR

Total RNA was isolated from HMC-1 according to the manufacturer's specifications using an easy-BLUE RNA extraction kit (iNtRON Biotech, Korea). Total RNA (2.0 μg) was heated at 65 °C for 10 min and then chilled on ice. Each sample was reverse-transcribed to cDNA for 90 min at 37 °C using a cDNA synthesis kit. RT-PCR was carried out with 1 μl of a cDNA mixture, in 20 μl final volume with 2.5 mM $MgCl_2$, 200 mM dNTPs, 25 pM cytokine primers, and 2.5 U of TaqDNA polymerase in the reaction buffer (50 mM KCl, 10 mM Tris–HCl, pH 9, and 0.1% Triton X-100). PCR was performed with the following primers for human IL-1β (5′ CCG GAT CCA TGG CAC CTG TAC GAT CA 3′; 5′ GGG GTA CCT TAG GAA GAC ACA AAT TG 3′); human IL-6 (5′ GAT GGA TGC TTC CAATCT GGAT 3′; 5′ AGT TCT CCATAG AGA ACA ACA TA 3′); human TNF-α (5′ CAC CAG CTG GTT ATC TCT CAG CTC 3′; 5′ CGG GAC GTG GAG CTG GCC GAG GAG 3′); human GAPDH (5′ CAA AAG GGT CAT CAT CTC TG 3′; 5′ CCT GCT TCA CCA CCT TCT TG 3′). The annealing temperatures used were 50 °C for IL-1β, 56 °C for IL-6, and 60 °C for TNF-α and GAPDH. Products were electrophoresed on a 1.5% agarose gel and visualized by ethidium bromide staining.

2.8. Western blot analysis

For determine protein levels, stimulated cells were rinsed twice with ice-cold phosphate buffered saline (PBS) and then lysed in ice-cold lysis buffer (PBS containing 0.1% SDS, 1% triton and 1% deoxycholate). Cell lysates were separated through electrophoresis, the protein was transferred to nylon membranes by electrophoretic transfer. The membranes were blocked in 6% skim milk for 2 h, rinsed and incubated overnight at 4 °C with primary Abs. After three washes in PBS containing 0.05% Tween-20 (PBST), the membranes were incubated for 1 h with horse radish peroxidase-conjugated secondary Abs. After three washes in PBST, the protein bands were visualized by an enhanced chemiluminescence assay following the manufacturer's instructions.

2.9. Caspase-1 activity determination

The enzymatic activity of caspase-1 was assayed using a caspase-1 colorimetric assay kit according to the manufacturer's protocol. The lysed cells were centrifuged at 15,000 × *g* for 5 min.

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