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Copper oxide nanoparticle induces inflammatory response and mucus production via MAPK signaling in human bronchial epithelial cells

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ABSTRACT

Copper nanoparticles (CuONPs) can pose risks to industrial workers. With increase of its applications especially in electronic fields, it is necessary to assess the toxicity of CuONPs, including pulmonary toxicity. In this study, we investigated the effect of CuONPs on human epithelial cell line H292. CuONPs treatment caused a significant increase in IL-6 and IL-8 mRNA expression and protein levels in H292 cells in a concentration-dependent manner. The mRNA expression and protein levels of MUC5AC were consistent with those of proinflammatory mediators. Additionally, CuONPs treatment increased phosphorylation of mitogen-activated protein kinases (MAPKs), Erk, JNK, and p-38 compared to that of control in a concentration-dependent manner. However, co-treatment with CuONPs and each MAPK inhibitor significantly decreased the phosphorylation of each MAPK, resulting in decreased mRNA expression and protein levels of proinflammatory mediators and MUC5AC compared to that in H292 cells only treated with CuONPs. In summary, CuONPs-induced inflammatory mediators and MUC5AC associated with MAPKs phosphorylation. Our results will provide useful information on CuONPs-induced pulmonary toxicity.

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1. Introduction

Nanotechnology using nanoparticles has increased rapidly in the past decades. Nanoparticles are used in many industries such as electronics, drug delivery, cosmetics, and clothing (Tiede et al., 2015; Anik et al., 2013). Currently, many researchers have investigated the potential toxicity of nanoparticles using various experiments, including a toxicological assessment study (Elsaesser and Howard, 2012). It has been reported that nanoparticle treatment can produce reactive oxygen species (ROS) that directly induces oxidative stress and indirectly mediates apoptosis (Donaldson and Poland, 2012). In addition, nanoparticles can cause DNA adduct formation and affect the expression of pro-inflammatory genes (Donaldson and Poland, 2012). According to Goncalves and Girard (2011), TiO_2 nanoparticles can elevate pro-inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and IL-6. In addition, mitogen-activated protein kinases (MAPKs), nuclear factor-κB (NF-κB) and adaptor protein-1 (AP-1) transcription factors are activated in cells exposed to nanoparticles (Piret et al., 2012).

Copper nanoparticles (CuONPs) as metal based nanoparticles have wide applications. They can be used in semiconductors, electronic chips, and heat transfer nanofluids (Mortimer et al., 2010). With their wide applications, toxicity studies of CuONPs have been performed in various experimental models. Recently, Piret et al. (2012) have reported that CuONPs can induce hepatotoxicity. In addition, CuONPs can induce oxidative stress resulting in DNA damage, mitochondrial dysfunction, and production of proinflammatory mediators (Nel et al., 2006; Piret et al., 2012; Bondarenko et al., 2013). The common exposure route of CuONPs is inhalation (Oberdorster and Utell, 2002; Li et al., 2010). Therefore, it is necessary to investigate pulmonary toxicity of CuONPs. Some

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experiments have demonstrated the pulmonary toxicity of CuONPs (Jeong et al., 2015; Isama, 2014). It has been reported that seven young female workers who were exposed to nanoparticles via inhalation for 5–13 months have suffered from shortness of breath and pleural effusion (Song et al., 2009). Limited pulmonary toxicity data and its underlying mechanisms induced by CuONPs are available. Therefore, we investigated the potential pulmonary toxicity of CuONPs by using CuONPs-treated human airway epithelial cell line NCI-H292 in this study. We also evaluated the levels of pro-inflammatory mediators and MUC5AC because MUC5AC is a major source of gel-forming mucin among human mucin gene family (Gipson, 2004). In addition, we investigated the underlying mechanisms of CuONPs-induced inflammatory response and mucus production. Furthermore, we evaluated the expression levels of MAPKs in CuONPs-treated NCI-H292 cells.

2. Materials and methods

2.1. Cell culture

Human airway epithelial cell line NCI-H292 was obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) in the presence of penicillin (100 U/mL), streptomycin (100 μg/mL) and HEPES (25 mM). Cells were incubated in a 5% CO_2 incubator at 37 °C.

2.2. Cytotoxicity assay

The effect of various experimental modulations on cell viability were evaluated by determining mitochondrial reductase function based on the reduction of tetrazolium salt, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, CAS#298-93-1; AMRESCO, Solon, OH), into formazan crystals. The formation of formazan is proportional to the number of functional mitochondria in living cells. To determine cell viability, cells were seeded into 96-well plates at a density of 5×10^5 cells/well and incubated in serum-free medium in the presence of varying concentrations of CuONPs (544868, Sigma-Aldrich, St. Louis, MO). The size of CuONPs was less than 50 nm. After 24 h incubation, 50 mg/mL of MTT was added to 5 μL of cell suspension (5×10^5 cells/mL in 96-well plates) for 4 h. The formazan formed was dissolved in acidic 2-propanol. Optical density was measured using a microplate reader (Benchmark; Bio-Rad Laboratories, Hercules, CA) at 570 nm. The optical density of formazan formed in control (untreated) cells was considered to have 100% viability. All experiments were performed in triplicates.

2.3. Effect of CuONPs on pro-inflammatory mediators mRNA expression and protein levels in H292 cells

Cells were seeded into 12-well plates at a density of 5×10^5 cells/well and treated with a nontoxic concentration of CuONPs (0.25, 0.5, 1, and 2 μg/mL, respectively). Cells were harvested after 24 h of incubation. Total RNA was isolated using Trizol™ reagent (Invitrogen, Carlsbad, CA) as instructed by the manufacturer. Reverse transcription reaction was performed using a cDNA synthesis kit (Qiagen, Hilden, Germany). Reverse-transcription polymerase chain reaction (RT-PCR) was used to measure the mRNA expression levels of pro-inflammatory mediators. In RT-PCR, polymerase chain reactions were performed using gene-specific forward and reverse primers (IL-6 forward; 5′-GAC AGC CAC TCA CCT CTT CA-3′, IL-6 reverse; 5′-AGT GCC TCT TTG CTG CTT TC-3′; IL-8 forward; 5′-ATG ACT TCC AAG CTG GCC GTG GCT-3′, IL-8 reverse; 5′-TTA TGA ATT CTC AGC CCT CTT CAA AAA-3′; β-actin forward; 5′-CAT GTA CGT TGC TAT CCA GGC-3′, β-actin reverse; 5′-CTC CTT AAT GTC ACG CAC GAT-3′) according to the manufacturer's instructions (Bioneer, Daejeon, Korea). The protein levels of IL-6 (BD Biosciences, San Jose, CA) and IL-8 (BD Biosciences) were measured using an enzyme-linked immunosorbent assay (ELISA) kit. The absorbance was measured at 450 nm using a microplate reader (Bio-Rad Laboratories, Hercules, CA).

2.4. Effect of CuONPs on MUC5AC mRNA expression and protein levels in H292 cells

Cells were seeded into 12-well plates at a density of 5×10^5 cells/well, treated with a nontoxic concentration of CuONPs (0.25, 0.5, 1 and 2 μg/mL), and harvested after 24 h. RT-PCR was performed to measure the mRNA expression of MUC5AC. Total RNA was isolated using Trizol™ reagent (Invitrogen, Carlsbad, CA). Reverse transcription reaction was performed using a cDNA synthesis kit (Qiagen, Hilden, Germany). In RT-PCR, polymerase chain reactions were performed using specific forward and reverse primers (MUC5AC forward; 5′-TGA TCA TCC AGC AGC AGG GCT-3′, MUC5AC reverse; 5′-CCG AGC TCA GAG GAC ATA TGGG-3‘; β-actin forward; 5′-CAT GTA CGT TGC TAT CCA GGC-3′, β-actin reverse; 5′-CTC CTT AAT GTC ACG CAC GAT-3′) according to the manufacturer's instructions (Bioneer, Daejeon, Korea). Protein levels of MUC5AC (MyBioSource, San Diego, CA) were determined using an ELISA kit. The absorbance was measured at 450 nm using a microplate reader (Bio-Rad Laboratories, Hercules, CA).

2.5. Effect of CuONPs on the expression of MAPKs in H292 cells

Cells were treated with various concentrations of CuONPs and then incubated for 1 h. Cells were collected via centrifugation, washed twice with PBS, and resuspended in cell extraction lysis buffer (Sigma-Aldrich) containing protease inhibitors. Protein concentration was determined using a protein assay reagent (Bio-Rad Laboratories) according to the manufacturer's instructions. Equal amounts of total cellular protein (30 μg) were resolved using 10% SDS-polyacrylamide gel electrophoresis, transferred to polyvinyl difluoride (PVDF) membrane, blocked with 5% skim milk for 1 h and incubated overnight at 4 °C with appropriate primary antibodies, including Erk (1:2000 dilution; Cell Signaling, Danvers, MA), p-Erk (1:1000 dilution; Cell Signaling), JNK (1:1000 dilution; Santa Cruz, Dallas, TX), p-JNK (1:1000 dilution; Santa Cruz), p38 (1:1000 dilution; Santa Cruz), p-p38 (1:1000 dilution; Santa Cruz) and β-actin (1:1000 dilution; Cell Signaling). Protein blots were washed three times with Tris-buffered saline containing 0.05% Tween 20 (TBST) followed by incubation with 1:3000 dilution of horseradish peroxidase (HRP)-conjugated secondary antibody (Jackson Immuno-Research, West Grove, PA) for 30 min at room temperature. Protein blots were washed again three times with TBST. Protein bands were developed using an enhanced chemiluminescence (ECL) kit (Thermo Scientific, Waltham, MA). Densitometric analysis for each protein band was determined using Chemi-Doc (Bio-Rad Laboratories).

2.6. Effects of CuONPs on MAPKs signaling in H292 cells

To investigate the effect of CuONPs on MAPK signaling, cells were pretreated with CuONPs (2 μg/mL) and MAPK inhibitors, PD098059 (Erk inhibitor, 20 μM, Millipore Co., Bedford, MA), SP600125 (JNK inhibitor, 20 μM, Millipore Co.) and SB203580 (p-38 inhibitor, 10 μM, Millipore Co.). After incubation for 1 h or 24 h, The MAPK signaling was investigated via Western blots as described above.

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