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Cytotoxicity, permeability, and inflammation of metal oxide nanoparticles in human cardiac microvascular endothelial cells

Cytotoxicity, permeability, and inflammation of metal oxide nanoparticles

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Abstract Wide applications and extreme potential of metal oxide nanoparticles (NPs) increase occupational and public exposure and may yield extraordinary hazards for human health. Exposure to NPs has a risk for dysfunction of the vascular endothelial cells. The objective of this study was to assess the cytotoxicity of six metal oxide NPs to human cardiac microvascular endothelial cells (HCMECs) in vitro. Metal oxide NPs used in this study included zinc oxide (ZnO), iron(III) oxide (Fe_2O_3), iron(II,III) oxide (Fe_3O_4), magnesium oxide (MgO), aluminum oxide (Al_2O_3), and copper(II) oxide (CuO). The cell viability, membrane leakage of lactate dehydrogenase, intracellular reactive oxygen species, permeability of plasma membrane, and expression of inflammatory markers vascular cell adhesion molecule-1, intercellular adhesion molecule-1, macrophage cationic peptide-1, and interleukin-8 in HCMECs were assessed under controlled and exposed conditions (12–24 h and 0.001–100 μg/ml of exposure). The results indicated that Fe_2O_3, Fe_3O_4, and Al_2O_3 NPs did not have significant effects on cytotoxicity, permeability, and inflammation response in HCMECs at any of the concentrations tested. ZnO, CuO, and MgO NPs produced the cytotoxicity at the concentration-dependent and time-dependent manner, and elicited the permeability and inflammation response in HCMECs. These results demonstrated that cytotoxicity, permeability, and inflammation in vascular endothelial cells following exposure to metal oxide nanoparticles depended on particle composition, concentration, and exposure time.

Keywords Cytotoxicity · Permeability · Inflammation · Metal oxide nanoparticles · Vascular endothelial cells

Abbreviations

NPs	Nanoparticles
Fe_2O_3	Iron(III) oxide
Y_2O_3	Yttrium oxide
CeO_2	Cerium oxide
ZnO	Zinc oxide
Fe_3O_4	Iron(II,III) oxide

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MgO	Magnesium oxide
Al_2O_3	Aluminum oxide
CuO	Copper(II) oxide
ECM	Endothelial cell medium
HCMECs	Human cardiac microvascular endothelial cells
LDH	Lactate dehydrogenase
ROS	Reactive oxygen species
VCAM-1	Vascular cell adhesion molecule-1
ICAM-1	Intercellular adhesion molecule 1
MCP-1	Macrophage cationic peptide-1
IL-8	Interleukin-8

Introduction

Nanoparticles (NPs) are often defined as intentionally manufactured particles typically ranging from 1 to ~100 nm in diameter (Oberdorster et al. 2005). Because of the various applications and the extreme potential of metal oxide NPs in cosmetics, electronics, and medical fields, occupational and public exposure may dramatically increase in the future and yield extraordinary hazards for human health (Savage et al. 2007). Therefore, it is necessary to systematical evaluate the beneficial and cytotoxic effects of such the metal oxide NPs in biological systems.

Vascular endothelial cells play a central role in angiogenesis, carcinogenesis, atherosclerosis, myocardial infarction, limb and cardiac ischemia, and tumor growth (Houle and Huot 2006; Packard and Libby 2008). Previous studies have reported that metal oxide nanoparticles can lead to cytotoxic effects on vascular endothelial cells (Gojova et al. 2007, 2009; Kennedy et al. 2009; Rosas-Hernandez et al. 2009; Yu et al. 2010; Chen et al. 2008b). The results of these studies demonstrate that cytotoxicity and inflammation in vascular endothelial cells after acute exposure to metal oxide NPs depend on the concentration and composition of the particles. Iron(III) oxide (Fe_2O_3), yttrium oxide (Y_2O_3), cerium oxide (CeO_2), and zinc oxide (ZnO) NPs were all internalized into human aortic endothelial cells, but only Y_2O_3 and ZnO elicited a pronounced inflammatory response (Kennedy et al. 2009; Gojova et al. 2009, 2007). Exposure to Fe_2O_3 NPs induces an increase in human microvascular endothelial cell permeability through reactive oxygen species (ROS) oxidative stress-modulated microtubule remodeling (Apopa et al. 2009). Therefore, a more thorough understanding of the potential biological effects of different components of NPs on vascular endothelial cells is required.

The main goal of the present work was to investigate the cytotoxicity, permeability, and inflammation in human cardiac microvascular endothelial cells (HCMECs) exposed to six different metal oxide NPs, including ZnO, Fe_2O_3, iron(II,III) oxide (Fe_3O_4), magnesium oxide (MgO), aluminum oxide (Al_2O_3), and copper(II) oxide (CuO). The cell viability, membrane leakage of lactate dehydrogenase (LDH), intracellular ROS, permeability of plasma membrane, and expression of inflammatory markers vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), macrophage cationic peptide-1 (MCP-1), and interleukin-8 (IL-8) in HCMECs were assessed under controlled and exposed conditions (12–24 h and 0.001–100 μg/ml of exposure).

Materials and methods

Nanoparticles preparation

ZnO (Cat. no. 677450, >97%), Fe_2O_3 (Cat. no. 544884), Fe_3O_4 (Cat. no. 637106, ≥ 98% trace metals basis), MgO (Cat. no. 549649), Al_2O_3 (Cat. no. 544833), and CuO nanopowders (Cat. no. 544868) were all purchased from Sigma-Aldrich (St. Louis, MO).

NPs were weighed to glass tubes and sterilized by heating for 4 h at 180°C in the oven, and then suspended in sterile saline (0.9% NaCl) in a sealed conical flask. The suspension stirred at the ambient temperature overnight and formed 5,000 μg/mL stock solution. In order to break the agglomerate and ensure a uniform suspension, all NPs were sonicated for 30 min with 50 W (JY99-IID, Ningbo Scientz Biotechnology Co., Ltd., China). The final serial dilutions of stock solutions in the completed endothelial cell medium (ECM; ScienCell, Carlsbad, CA) were performed. The characteristics of NPs were measured at 50 μg/ml. Morphology of NPs was examined by transmission electron microscopy (Hitachi H-7600, Japan), and then the equivalent spherical diameter of NPs was calculated. Specific surface area of NPs was quantified by Brunauer, Emmett, and Telle method (Micromeritics Tristar 3000, USA). The

compositions of NPs were quantitatively analyzed by Raman spectroscopic technique (RW-1000, UK). The zeta potential and particle diameter of NPs were measured by the Malvern Instruments' Zetasizer systems (Malvern Zetasize 3000HSA, UK). The characteristics of NPs were shown in Table 1.

Cell culture

HCMECs were purchased from ScienCell. The cells were cultured according to the protocol described previously (Dossumbekova et al. 2008). Briefly, HCMECs were grown in completed ECM. The cells were maintained in an incubator at 37°C with 5% CO_2.

Nanoparticles treatment

HCMECs were seeded in 100-mm cell culture plates at 1×10^6 cells/plate in a total volume of 10 ml. When confluent, cells were trypsinized by 0.25% Trypsin-0.02% EDTA (Invitrogen), and seed in 96-well plates at 5×10^4 cells/well (total volume of 200 μl/well) and in 60-mm culture plate at 5×10^5 cells/plate (total volume 5 ml/plate), respectively. Twelve hours after seeding, cells were washed three times with ECM without antibiotics, and the ECM supplemented with appropriate concentrations (0.001, 0.01, 0.1, 1, 5, 10, 20, 50, and 100 μg/ml) of NPs or ECM alone was applied to cells. The cells were exposed to NPs for 12 or 24 h.

Cell viability assays

Effects of NPs on the cell viability of HCMECs were evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Sigma) according to the protocol described previously (Mosmann 1983). HCMECs were plated into the 96-well plates and exposed to NPs as described previously. At the end of exposure, 20 μl MTT (0.5 mg/ml) was added to each well and incubated at 37°C for 4 h. The cell culture medium was aspirated cautiously, and 150 μl dimethyl sulfoxide was added to each well and mixed thoroughly. Optical density of each well was measured at 570 nm using ELISA reader (Wellscans MK3, Thermo Labsystems, Finland). All experiments were performed in triplicate.

Lactate dehydrogenase leakage assay

The leakage of LDH in HCMECs was determined using a LDH assay (Sigma) according to the manufacturer. HCMECs were plated into the 96-well plates and exposure in NPs as described previously. At the end of exposure, the aliquot of 50 μl cell medium was used for LDH activity analysis, and the absorption was measured using the ELISA reader at 490 nm. All experiments were performed in triplicate.

Reactive oxygen species detection

The ROS production was measured using flow cytometry according to the methods described previously (Chen et al. 2008a). HCMECs were plated into the 60-mm plates at 5×10^5 cells/plate and then incubated with 10 μM 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA; Invitrogen) for 60 min at 37°C. At the end of the incubation, cells were washed again

Table 1 Characteristics of nanoparticles used in the study

Particle name	Average diameter in TEM (nm)	Specific surface area (m^2/g)	Chemical composition	Average diameter in DLS (nm)	Zeta potential ζ (mV)	pH
ZnO	45.3	21.3	Zn, O	310	32.7	7.4
Fe_2O_3	42.5	82.8	Fe, O	957	−8.2	7.4
Fe_3O_4	46.8	147.4	Fe, O	1025	1.7	7.4
MgO	39.2	37.9	Mg, O	639	11.6	7.4
Al_2O_3	39.7	64.7	Al, O	267	36.3	7.4
CuO	46.3	24.1	Cu, O	239	42.8	7.4

The concentration of nanoparticles in the completed endothelial cell medium was 50 μg/ml. The equivalent spherical diameter was examined and calculated by transmission electron microscopy. Specific surface area was quantified by BET (Brunauer, Emmett, and Telle) method. Compositions were quantitatively analyzed by Raman spectroscopic technique. The zeta potential and particle diameter were measured by the Malvern Instruments' Zetasizer systems

with phosphate-buffered saline (PBS) and exposure in NPs as described previously. At the end of exposure, cells were quenched on ice for 10 min then washed three times with ice-cold PBS before they were harvested by scrapping. The cells were fixed with 10% formaldehyde for 20 min at room temperature and then washed three times with PBS, followed by resuspension in 400 ml of PBS. ROS measurements were carried out by a flow cytometry using FACS-Calibur system (BD Biosciences, Rutherford, NJ) with a 488-nm excitation beam. The signals were obtained using a 530-nm band-pass filter for CM-H2DCFDA. Each measurement was based on the mean fluorescence intensity of 1×10^4 cells. All experiments were performed in triplicate.

HCMECs permeability assay

HCMECs permeability was measured as described previously (Imai-Sasaki et al. 1995). HCMECs were cultured on 0.4-μm-pore-size mesh plate inserts (Millcell-CM, Millipore, MA, USA). Chambers were examined microscopically for confluence, integrity, and uniformity of HCMECs monolayers. Then, 25 μM of bovine serum albumin-fluorescein isothiocyanate conjugate (BSA-FITC) containing 2% BSA (Sigma) was added into the apical chamber of the inserts. The volumes used equalized fluid heights in the apical and basolateral chamber, so that only diffusive forces were involved in solute permeability. Then HCMECs were exposed to NPs as described previously. At the end of exposure, the medium was collected from the basolateral chamber. The concentration of BSA-FITC was determined by a fluorescence spectrophotometer. All experiments were performed in triplicate.

RNA isolation, reverse transcription, and quantitative real-time polymerase chain reaction analysis

Total RNA was extracted from HCMECs using TRIZOL Reagent (Invitrogen) according to the manufacturer's instruction. cDNAs were prepared from 1 μg total RNA using the reverse transcriptase enzyme SuperScript II (Invitrogen) according to the manufacturer's instructions. Real-time reverse-transcription polymerase chain reaction (RT-PCR) was performed using SYBR Green and an ABI 7000 sequence detection system (Applied Biosystems, Foster City, CA). These basic PCR amplification conditions were 58°C annealing temperature and 35 cycles. The gene primer sequences were used in this study:

Human VCAM-1: sense 5′-CAAATCCTTGA TACTGCTCATC-3′, antisense 5′-TTGACTTC TTGCTCACAGC-3′
ICAM-1: sense 5′-GCTATGCCTTGTCCTCTTG-3′, antisense 5′-ATACACACACACACACACA CGC-3′
MCP-1: sense 5′-CAGCCAGATGCAATCAATG-C-3′, antisense 5′-GTGGTCCATGGAATCCT GAA-3′
IL-8: sense 5′-AAACCACCGGAAGGAACCAT-3′, antisense 5′-CCTTCACACAGAGCTGCAG AAA-3′
18S rRNA: sense 5′-TAGAGTGTTCAAAGCA GGCCC-3′, antisense 5′-CCAACAAAATAGAA CCGCGGT-3′

Statistical analysis

The data were represented as means±standard deviation of three independent experiments. Statistical analysis of the data was carried out using one-way analysis of variance for multiple comparisons and independent-sample Student's t test for two-group comparisons. A value of $P<0.05$ was considered significant.

Results

Cytotoxicity of metal oxide nanoparticles on human cardiac microvascular endothelial cells

To assess the cytotoxicity of six metal oxide NPs (ZnO, Fe_2O_3, Fe_3O_4, MgO, Al_2O_3, and CuO), HCMECs were exposed to different concentrations (ranging from 0.001 to 100 μg/ml) of these NPs for 12 and 24 h. Cytotoxicity of ZnO, CuO, and MgO NPs was the concentration-dependent and time-dependent increase in HCMECs (Fig. 1). The results of MTT assays showed that ZnO and CuO exposure exhibited a significant cytotoxicity from 10 to 100 μg/ml for 12 h and from 5 to 100 μg/ml for 24 h. MgO NPs showed toxicity at 100 μg/ml for 12 and 50 μg/ml for 24-h exposures. In contrast, Fe_2O_3, Fe_3O_4, and Al_2O_3 NPs did not produce a significant increase in HCMECs death at the concentrations tested. More-

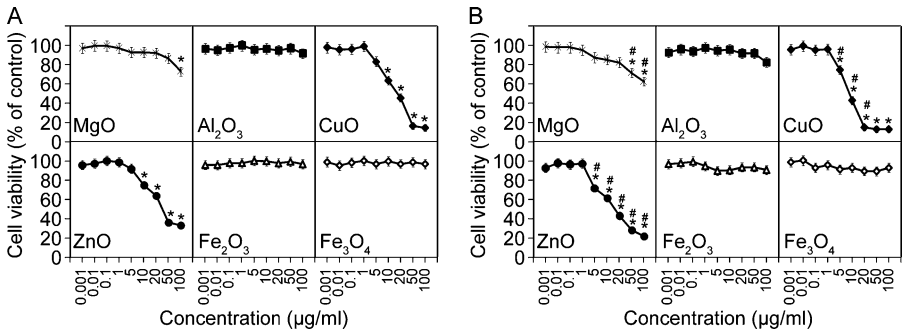

Fig. 1 Viability of human cardiac microvascular endothelial cells exposed to nanoparticles with different exposure concentrations determined by the MTT assay. Cells were respectively treated with 0.001–100 μg/ml of ZnO, Fe_2O_3, Fe_3O_4, MgO, Al_2O_3, or CuO nanoparticles for 12 (**a**) or 24 h (**b**). The viability was measured with the MTT assay. The results are given in percent related to untreated controls. Results are the mean±standard deviation (SD; *vertical bars*) of three independent experiments each carried out in triplicate. *Asterisk* indicates a statistically significant difference compared to untreated controls ($P<0.05$). *Number sign* indicates a statistically significant difference compared to the respective values for 12- and 24-h exposure ($P<0.05$)

over, there was an increase in cytotoxicity at the 5–100 μg/ml of ZnO, 5–20 μg/ml of CuO, and 50–100 μg/ml of MgO NPs exposure for 24 h compared to exposure for 12 h. The MTT assay showed that HCMECs were the most sensitive to CuO, then to ZnO, and finally, to MgO NPs.

LDH leakage in human cardiac microvascular endothelial cells exposed to metal oxide nanoparticles

LDH values from various concentrations of six different particle types at two different time points were compared with controls, which revealed the impact of NPs on cell membrane integrity (Fig. 2). The results demonstrated that exposure to ZnO, CuO, or MgO NPs for 12 and 24 h resulted in the concentration-dependent and time-dependent increase in LDH leakage. ZnO and CuO NPs exhibited the significant ($P<0.05$) increase of LDH leakage at 10–100 μg/ml for 12 h and 5–100 μg/ml for 24 h. The results of LDH leakage for MgO NPs exposure produced a significant effect at the concentration of 50 and 100 μg/ml for 12 and 24 h. On the contrary, the slight increase in LDH leakage was observed with Fe_2O_3, Fe_3O_4, and Al_2O_3 NPs at any of the concentrations tested. Moreover, there were significant differences when comparing the LDH leakage between different exposure times of 5, 50, and

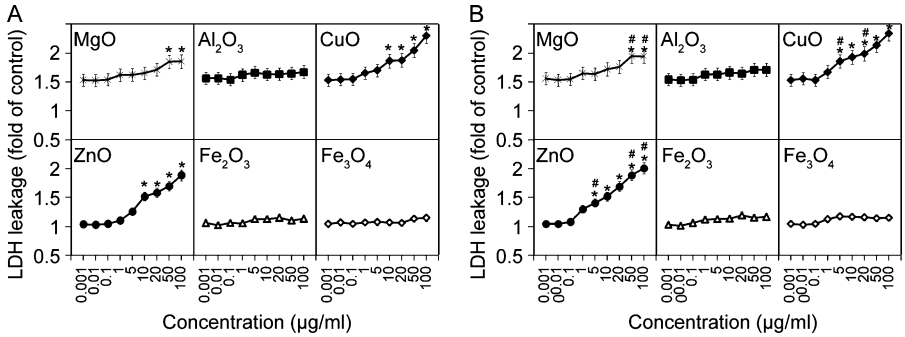

Fig. 2 Effect of nanoparticles on lactate dehydrogenase (LDH) leakage in human cardiac microvascular endothelial cells. Cells were respectively treated with 0.001–100 μg/ml of ZnO, Fe_2O_3, Fe_3O_4, MgO, Al_2O_3, or CuO nanoparticles for 12 (**a**) or 24 h (**b**). The values of LDH activity are expressed as fold increase relative to untreated controls. Results are the mean ±standard deviation (SD; *vertical bars*) of three independent experiments each carried out in triplicate. *Asterisk* indicates a statistically significant difference compared to untreated controls ($P<0.05$). *Number sign* indicates a statistically significant difference compared to the respective values for 12 and 24-h exposure ($P<0.05$)

100 μg/ml of ZnO NPs, 5 and 20 μg/ml of CuO NPs, and 50 and 100 μg/ml of MgO NPs. Based on LDH results, ZnO, CuO, and MgO NPs had the more significant cytotoxicity when compared to other particles.

Reactive oxygen species generation in human cardiac microvascular endothelial cells exposed to metal oxide nanoparticles

To investigate the potential role of oxidative stress as a mechanism of cytotoxicity of six metal oxide NPs, the effects produced on ROS was examined. Intracellular ROS was determined using CM-H_2DCFDA. ROS generation following 12 and 24 h of exposure to six NPs at 0.001–100 μg/ml is shown in Fig. 3. The results showed that the level of ROS in cells increased in a concentration-dependent and time-dependent manner after exposure to ZnO, CuO, or MgO NPs for 12 and 24 h. The results of ROS generation for ZnO and CuO NPs exposure produced a significant effect from 20 μg/ml concentration for 12 h and 5 μg/ml for 24 h. MgO NPs treatment at 50 and 100 μg/ml for 12 h and 20, 50, and 100 μg/ml for 24 h resulted in the statistical increase of fluorescence intensity. In contrast, Fe_2O_3, Fe_3O_4, and Al_2O_3 NPs did not have significant effects on the generation of ROS in HCMECs at the concentrations tested. Moreover, there was an increase in ROS generation at the 5–100 μg/ml of ZnO, 5–20 μg/ml of CuO, and 20–100 μg/ml of MgO NPs exposure for 24 h compare to exposure for 12 h.

Permeability of human cardiac microvascular endothelial cells exposed to metal oxide nanoparticles

The permeability of HCMECs induced by six metal oxide NPs was assessed using BSA-FITC flux through endothelial monolayers (Fig. 4). The results demonstrated that ZnO and CuO NPs significantly increased the permeability of HCMECs at the concentration-dependent manner from 20 to 100 μg/ml concentration for 12 and 24 h. The results of permeability for MgO NPs exposure produced a significant increase at the concentration of 100 μg/ml for 12 and 24 h. In contrast, treatment of HCMECs for 12 and 24 h with Fe_2O_3, Fe_3O_4, and Al_2O_3 NPs failed to lead to an increase in endothelial permeability.

Effect of nanoparticles on human cardiac microvascular endothelial cells inflammatory

In order to investigate the inflammatory potential of six NPs on HCMECs, the mRNA expression of VCAM-1, ICAM-1, MCP-1, and IL-8 for 12 and 24 h of treatment was examined. ZnO and CuP NPs induced the significantly increased production of these inflammatory markers at the concentration from 5 μg/ml for 12- and 24-h exposures (Fig. 5) at the concentration-dependent manner. For instance, at 100 μg/ml for 24-h exposure, ZnO NPs increased VCAM-1, ICAM-1, MCP-1, and IL-8 mRNA levels relative to control cells by about 8.662, 8.264, 9.071, and 6.472 times, respectively ($P<0.05$ for all markers). MgO NPs also induced significantly higher

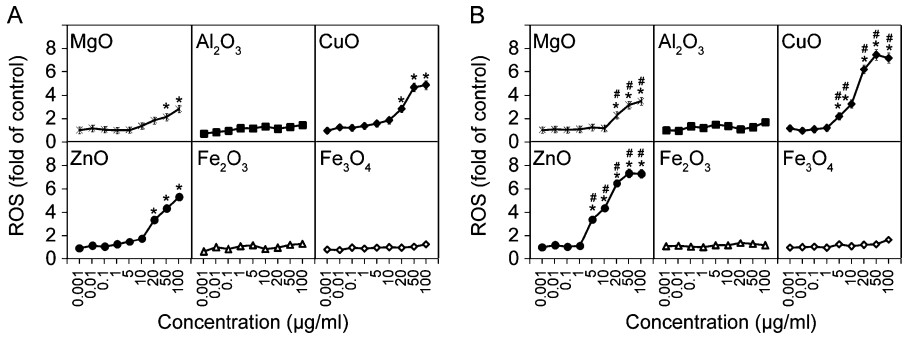

Fig. 3 Effect of nanoparticles on reactive oxygen species (ROS) generation in human cardiac microvascular endothelial cells. Cells were respectively treated with 0.001–100 μg/ml of ZnO, Fe_2O_3, Fe_3O_4, MgO, Al_2O_3, or CuO nanoparticles for 12 (**a**) or 24 h (**b**). Data are reported as fold increase in fluorescence intensity relative to untreated controls. Results are the mean±standard deviation (SD; *vertical bars*) of three independent experiments each carried out in triplicate. *Asterisk* indicates a statistically significant difference compared to untreated controls ($P<0.05$). *Number sign* indicates a statistically significant difference compared to respective values for 12- and 24-h exposure ($P<0.05$)

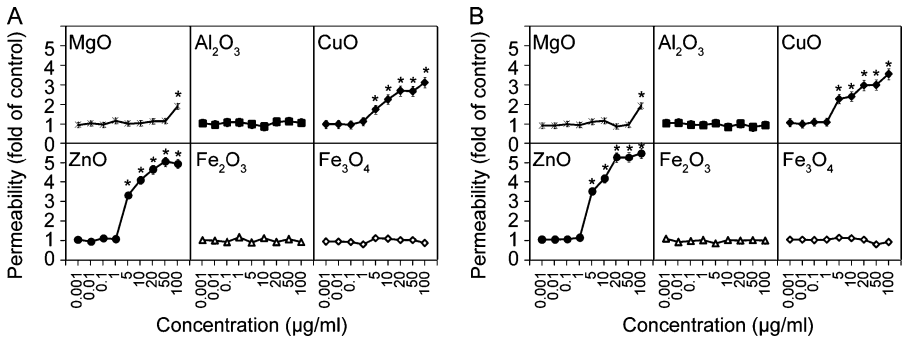

Fig. 4 Permeability of human cardiac microvascular endothelial cells after treatment with nanoparticles. Cells were respectively treated with 0.001–100 μg/ml of ZnO, Fe_2O_3, Fe_3O_4, MgO, Al_2O_3, or CuO nanoparticles for 12 (**a**) or 24 h (**b**). Data are reported as fold increase in fluorescence intensity relative to untreated controls. Results are the mean±standard deviation (SD; *vertical bars*) of three independent experiments each carried out in triplicate. *Asterisk* indicates a statistically significant difference compared to untreated controls ($P<0.05$)

levels of VCAM-1, ICAM-1, and MCP-1 at the 50 μg/ml, and IL-8 at the 100 μg/ml for 12 and 24-h exposures. At 100 μg/ml for 24 h, VCAM-1, ICAM-1, MCP-1, and IL-8 mRNA levels increased relative to control cells by about 4.737, 3.635, 4.869, and 2.541 times, respectively. In contrast, Fe_2O_3, Fe_3O_4, and Al_2O_3 NPs did not have any effects on the mRNA expression of these inflammatory markers in HCMECs.

Discussion

Previous studies give some insights regarding cytotoxicity and inflammatory of vascular endothelial cells induced by NPs (Gojova et al. 2007, 2009; Kennedy et al. 2009; Rosas-Hernandez et al. 2009), and the intimate metal oxide nanoparticle composition is a major determinant of propensity to induce the biological effects (Gojova et al. 2007; Kennedy et al. 2009). In this study, we studied the impact of cytotoxicity, permeability, and inflammation in human cardiac microvascular endothelial cells induced by six metal oxide nanoparticles at 2 time points and 9 concentration points ranging from 0.001 to 100 μg/ml.

This study showed that there was a high variation in the ability of nanoparticles to cause cytotoxic effects. The most important finding in this study was the high cytotoxicity and ability of ZnO, CuO, and MgO nanoparticles to cause oxidative lesions. Such results are in accordance with data from literature that reported the cytotoxicity induced by metal oxide NPs in vitro. In literature, ZnO NPs induced great cytotoxicity of human embryonic lung fibroblasts (Yuan et al. 2010), human colon carcinoma cells (De Berardis et al. 2010), and human bronchial epithelial cells (Heng et al. 2010). CuO NPs are highly cytotoxicity of human lung epithelial cell line A549 (Karlsson et al. 2008), whereas MgO NPs have the slight effective in inducing cell death in human astrocyte-like astrocytoma U87 cells (Lai et al. 2008).

The high cytotoxicity of ZnO, CuO, and MgO nanoparticles was in contrast to the Fe_2O_3, Fe_3O_4, and Al_2O_3 nanoparticles. These nanoparticles showed no or low toxic effects when human HCMECs were exposed. This is in agreement with the other studies showing low cytotoxic effects of iron oxide nanoparticles (Fahmy and Cormier 2009; Hussain et al. 2005; Veranth et al. 2007) and Al_2O_3 nanoparticles (Kim et al. 2010; Wang et al. 2009).

Numerous studies have shown that increase endothelial permeability related with ROS-induced oxidant stress is one of the major roles in angiogenic-related diseases (Lum and Roebuck 2001; Holman and Maier 1990). The treatment of endothelial cell monolayers with iron nanoparticles increases endothelial cell permeability, and the addition of H_2O_2 enhances iron nanoparticle-induced cell permeability, demonstrating that the production of ROS is involved in iron nanoparticle-induced permeability (Apopa et al. 2009). In our study, we revealed that the iron oxide (Fe_2O_3 and Fe_3O_4) nanoparticles are the least toxic nanomaterials among all the six nano-sized metal oxides. By comparison, ZnO, CuO, and MgO NPs significantly increased the permeability of HCMECs

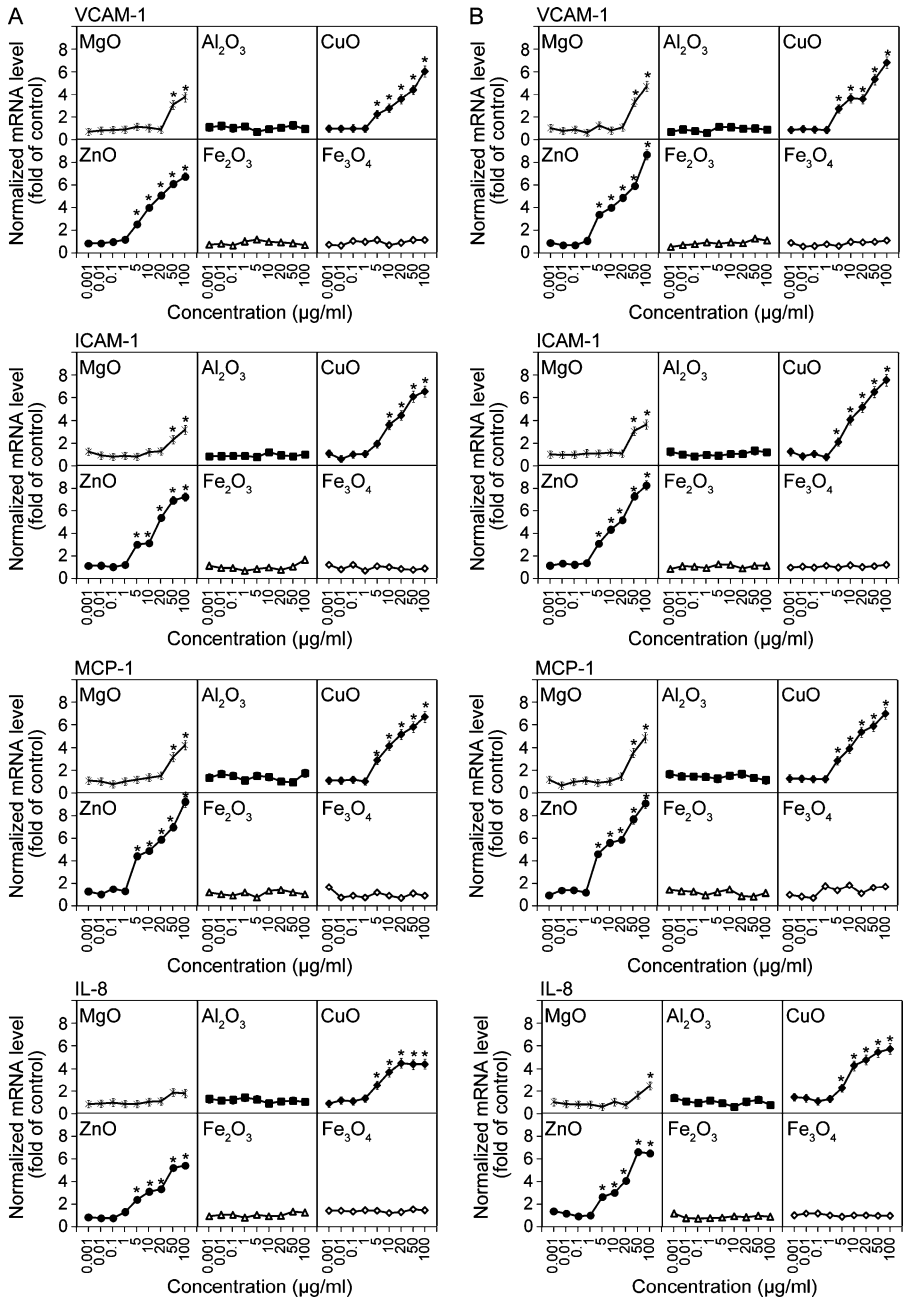

Fig. 5 Ratio of mRNA expressions of the inflammatory markers VCAM-1, ICAM-1, MCP-1, and IL-8 in human cardiac microvascular endothelial cells exposed to nanoparticles. Cells were respectively treated with 0.001–100 μg/ml of ZnO, Fe_2O_3, Fe_3O_4, MgO, Al_2O_3, or CuO nanoparticles for 12 (**a**) or 24 h (**b**). Each mRNA value was normalized to corresponding 18S rRNA value. Ratios were relative to untreated controls. Results are the mean±standard deviation (SD; *vertical bars*) of three independent experiments each carried out in triplicate. *Asterisk* indicates a statistically significant difference compared to untreated controls ($P<0.05$)

at the concentration-dependent manner. These results were consistent in the results of ROS generation.

During the inflammation process of the vein, the activation of endothelial cells is a crucial step. The activated endothelial cells significantly expressed the adhesion molecules ICAM-1 and VCAM-1 and chemokines IL-8 and MCP-1. Upregulation of adhesion molecules on the surface of endothelial cells promotes monocytes adhesion (Balciunas et al. 2009; van Buul and Hordijk 2008), while IL-8 and MCP-1 participate in recruiting monocytes into the subendothelial cell layer (Gerszten et al. 1999). Therefore, factors affecting the expression of endothelial adhesion molecules and chemokines are important in the regulation of the vascular inflammatory processes. In the present study, the stimulant effects of NP treatment on adhesion and inflammation molecule expression in HCMECs were observed. Our results

demonstrated that exposure of HCMECs to ZnO, CuO, or MgO nanoparticles significantly upregulated mRNA levels of the inflammatory markers VCAM-1, ICAM-1, MCP-1, and IL-8; whereas, Fe_2O_3, Fe_3O_4, and Al_2O_3 NPs had no effect. The inflammatory response did not initiate below the concentration of 5 μg/ml of ZnO and CuO or 100 μg/ml of MgO. Future investigations may provide the mRNA and protein expression of other related inflammation molecules to further clarify the molecular mechanism of NP-induced inflammation response of vascular endothelial cells. This result is similar to the report of Kennedy et al. (2009) that Fe_2O_3 nanoparticles did not provoke an inflammatory response in human aortic endothelial cells at any of the concentrations tested, and ZnO nanoparticles elicited a pronounced inflammatory response above a threshold concentration of 10 mg/ml.

In accordance with Gojova et al. (2007) and Hussain et al. (2009), our results revealed that cytotoxity, permeability, and inflammatory response of HCMECs appeared to not only correlate with the concentration and time exposure of NPs with different composition, but correlate inversely with the specific surface area of NPs. Fe_2O_3, Fe_3O_4, and Al_2O_3 NPs, which have the larger specific surface area among the six metal oxide NPs tested, had a slight effect on cytotoxity, permeability, and inflammatory response of HCMECs; whereas, ZnO, CuO, and MgO NPs have the smaller specific surface area and provoke the pronounced cytotoxity, permeability, and inflammatory response. Future investigations may provide more understanding of the relationship between surface properties and cellular uptake, translocation, metabolism, and other biological effects of different nanoparticles in vivo and in vitro.

Taken together, these results demonstrate that there was a high variation among different nanoparticles regarding their ability to cause cytotoxicity, permeability, and inflammation in human vascular endothelial cells. ZnO, CuO, and MgO nanoparticles were most potent, and the exposure to these particles may pose a health risk. Iron oxide particles (Fe3O4 and Fe2O3) showed no or low cytotoxicity. Results provided here may have implications for understanding the bioactivity of nanoparticles involving vascular diseases. The different toxicity according to particle composition could be an important concept of safety biomedical applications of NPs.

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Conflict of interest The authors have no conflict of interest.

References

Apopa PL, Qian Y, Shao R, Guo NL, Schwegler-Berry D, Pacurari M, et al. Iron oxide nanoparticles induce human microvascular endothelial cell permeability through reactive oxygen species production and microtubule remodeling. Part Fibre Toxicol. 2009;6:1.

Balciunas M, Bagdonaite L, Samalavicius R, Baublys A. Markers of endothelial dysfunction after cardiac surgery: soluble forms of vascular-1 and intercellular-1 adhesion molecules. Medicina (Kaunas). 2009;45(6):434–9.

Chen JR, Shankar K, Nagarajan S, Badger TM, Ronis MJ. Protective effects of estradiol on ethanol-induced bone loss involve inhibition of reactive oxygen species generation in osteoblasts and downstream activation of the extracellular signal-regulated kinase/signal transducer and activator of transcription 3/receptor activator of nuclear factor-kappaB ligand signaling cascade. J Pharmacol Exp Ther. 2008a;324 (1):50–9.

Chen L, Yokel RA, Hennig B, Toborek M. Manufactured aluminum oxide nanoparticles decrease expression of tight junction proteins in brain vasculature. J Neuroimmune Pharmacol. 2008b;3(4):286–95.

De Berardis B, Civitelli G, Condello M, Lista P, Pozzi R, Arancia G, et al. Exposure to ZnO nanoparticles induces oxidative stress and cytotoxicity in human colon carcinoma cells. Toxicol Appl Pharmacol. 2010;246(3):116–27.

Dossumbekova A, Berdyshev EV, Gorshkova I, Shao Z, Li C, Long P, et al. Vanden Hoek TL. Akt activates NOS3 and separately restores barrier integrity in H2O2-stressed human cardiac microvascular endothelium. Am J Physiol Heart Circ Physiol. 2008;295(6):H2417–26.

Fahmy B, Cormier SA. Copper oxide nanoparticles induce oxidative stress and cytotoxicity in airway epithelial cells. Toxicol In Vitro. 2009;23(7):1365–71.

Gerszten RE, Garcia-Zepeda EA, Lim YC, Yoshida M, Ding HA, Gimbrone Jr MA, et al. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. Nature. 1999;398(6729):718–23.

Gojova A, Guo B, Kota RS, Rutledge JC, Kennedy IM, Barakat AI. Induction of inflammation in vascular endothelial cells by metal oxide nanoparticles: effect of particle composition. Environ Health Perspect. 2007;115(3):403–9.

Gojova A, Lee JT, Jung HS, Guo B, Barakat AI, Kennedy IM. Effect of cerium oxide nanoparticles on inflammation in vascular endothelial cells. Inhal Toxicol. 2009;21 Suppl 1:123–30.

Heng BC, Zhao X, Xiong S, Ng KW, Boey FY, Loo JS. Toxicity of zinc oxide (ZnO) nanoparticles on human bronchial epithelial cells (BEAS-2B) is accentuated by oxidative stress. Food Chem Toxicol. 2010;48(6):1762–6.

Holman RG, Maier RV. Oxidant-induced endothelial leak correlates with decreased cellular energy levels. Am Rev Respir Dis. 1990;141(1):134–40.

Houle F, Huot J. Dysregulation of the endothelial cellular response to oxidative stress in cancer. Mol Carcinog. 2006;45(6):362–7.

Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlager JJ. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol In Vitro. 2005;19(7):975–83.

Hussain S, Boland S, Baeza-Squiban A, Hamel R, Thomassen LC, Martens JA, et al. Oxidative stress and proinflammatory effects of carbon black and titanium dioxide nanoparticles: role of particle surface area and internalized amount. Toxicology. 2009;260(1–3):142–9.

Imai-Sasaki R, Kainoh M, Ogawa Y, Ohmori E, Asai Y, Nakadate T. Inhibition by beraprost sodium of thrombin-induced increase in endothelial macromolecular permeability. Prostaglandins Leukot Essent Fatty Acids. 1995;53 (2):103–8.

Karlsson HL, Cronholm P, Gustafsson J, Moller L. Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. Chem Res Toxicol. 2008;21(9):1726–32.

Kennedy IM, Wilson D, Barakat AI. Uptake and inflammatory effects of nanoparticles in a human vascular endothelial cell line. Res Rep Health Eff Inst. 2009;136:3–32.

Kim IS, Baek M, Choi SJ. Comparative cytotoxicity of Al2O3, CeO2, TiO2 and ZnO nanoparticles to human lung cells. J Nanosci Nanotechnol. 2010;10(5):3453–8.

Lai JC, Lai MB, Jandhyam S, Dukhande VV, Bhushan A, Daniels CK, et al. Exposure to titanium dioxide and other metallic oxide nanoparticles induces cytotoxicity on human neural cells and fibroblasts. Int J Nanomedicine. 2008;3(4):533–45.

Lum H, Roebuck KA. Oxidant stress and endothelial cell dysfunction. Am J Physiol Cell Physiol. 2001;280(4): C719–41.

Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1–2):55–63.

Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect. 2005;113(7):823–39.

Packard RR, Libby P. Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. Clin Chem. 2008;54(1):24–38.

Rosas-Hernandez H, Jimenez-Badillo S, Martinez-Cuevas PP, Gracia-Espino E, Terrones H, Terrones M, et al. Effects of 45-nm silver nanoparticles on coronary endothelial cells and isolated rat aortic rings. Toxicol Lett. 2009;191(2–3):305–13.

Savage N, Thomas TA, Duncan JS. Nanotechnology applications and implications research supported by the US Environmental Protection Agency STAR grants program. J Environ Monit. 2007;9(10):1046–54.

van Buul JD, Hordijk PL. Endothelial signalling by Ig-like cell adhesion molecules. Transfus Clin Biol. 2008;15(1–2):3–6.

Veranth JM, Kaser EG, Veranth MM, Koch M, Yost GS. Cytokine responses of human lung cells (BEAS-2B) treated with micron-sized and nanoparticles of metal oxides compared to soil dusts. Part Fibre Toxicol. 2007;4:2.

Wang H, Wick RL, Xing B. Toxicity of nanoparticulate and bulk ZnO, Al2O3 and TiO2 to the nematode Caenorhabditis elegans. Environ Pollut. 2009;157(4):1171–7.

Yu M, Mo Y, Wan R, Chien S, Zhang X, Zhang Q. Regulation of plasminogen activator inhibitor-1 expression in endothelial cells with exposure to metal nanoparticles. Toxicol Lett. 2010;195(1):82–9.

Yuan JH, Chen Y, Zha HX, Song LJ, Li CY, Li JQ, et al. Determination, characterization and cytotoxicity on HELF cells of ZnO nanoparticles. Colloids Surf B Biointerfaces. 2010;76(1):145–50.