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Zinc oxide nanoparticles inhibit Ca^{2+}-ATPase expression in human lens epithelial cells under UVB irradiation

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ABSTRACT

Epidemiological and experimental studies have revealed that lens epithelial cells exposed to ultraviolet B (UVB) light could be induced apoptosis, and lens epithelial cell apoptosis can initiate cataractogenesis. Posterior capsular opacification (PCO), the most frequent complication after cataract surgery, is induced by the proliferation, differentiation, migration of lens epithelial cells. Thus, inhibiting the proliferation of lens epithelial cells could reduce the occurrence of PCO. It is reported that zinc oxide (ZnO) nanoparticles have great potential for the application of biomedical field including cancer treatment. In the present study, we investigated the cytotoxic effect of ZnO nanoparticles on human lens epithelial cell (HLEC) viability. In addition, changes in cell nuclei, apoptosis, reactive oxygen species and intracellular calcium ion levels were also investigated after cells treated with ZnO nanoparticles in the presence and absence of UVB irradiation. Meanwhile, the expression of plasma membrane calcium ATPase 1 (PMCA1) was also determined at gene and protein levels. The results indicate that ZnO nanoparticles and UVB irradiation have synergistic inhibitory effect on HLEC proliferation in a concentration-dependent manner. ZnO nanoparticles can increase the intracellular calcium ion level, disrupt the intracellular calcium homeostasis, and decrease the expression level of PMCA1. UVB irradiation can strengthen the effect of reduced expression of PMCA1, suggesting that both UVB irradiation and ZnO nanoparticles could exert inhibitory effect on HLECs *via* calcium-mediated signaling pathway. ZnO nanoparticles have great potential for the treatment of PCO under UVB irradiation.

1. Introduction

Higher energy wavelengths of ultraviolet light from sunlight including Ultraviolet B (UVB, 290–320 nm) are known to cause oxidative stress and ocular injury (Black et al., 2011; Kolozsvari et al., 2002; Podskochy, 2004). An increasing number of evidence indicate that chronic exposure of the eye to UVB irradiation may injure the lens, a process that causes aberrant epithelial cell growth and differentiation, as well as cell death *via* necrosis and apoptosis (Estil et al., 1997; Rogers et al., 2004), thereby forming cortical and posterior subcapsular cataract in humans and animals (Bochow et al., 1989; McCarty and Taylor, 1996; Michael et al., 2000; Wickert et al., 1999). At present, the most effective treatment of cataract is the surgical removal of the opacified lens and intraocular lens implantation. However, posterior capsular opacification (PCO), which involved in the lens epithelial cell proliferation and migration, is still one of the most common postoperative morbidities. Thus, inhibiting lens epithelial cell proliferation could efficiently reduce the occurrence of PCO after cataract surgery. Currently, the PCO is usually treated with YAG laser capsulotomy. However, this procedure can cause several complications including intraocular lens optic damage, postoperative intraocular pressure elevation and retinal detachment. Thus, there is an urgent need to prevent PCO.

Calcium ions (Ca^{2+}) can enter the cell through Ca^{2+} channels. Meanwhile, calcium ions can also be elevated *via* sarcoplasmic/endoplasmic reticulum *via* Ca^{2+}-release transporters. Thus, to keep the intracellular calcium homeostasis, components are needed which can delicate regulate the intracellular calcium ion level. The crucial components in regulating calcium homeostasis in cells include the Na^{+}/Ca^{2+} exchanger and plasma membrane calcium ATPase (PMCA) transporters, by which the intracellular calcium ions could be removed to maintain calcium homeostasis. For the regulation of intracellular sarcoplasmic calcium stores, it also

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could be achieved by sarcoplasmic/endoplasmic reticular calcium ATPase (SERCA). In human lens, cellular calcium homeostasis is acquired by a delicate balance of calcium ions among passive inward movement from the extracellular milieu through membrane channels (Cooper et al., 1986), extrusion by PMCA (Hightower and Kinsey, 1980) and internal sequestration by SERCA (Duncan et al., 1993). There are lens epithelial cells and fiber cells in human lens. Studies have substantiated that no calcium pump is located in the fiber cells (Marian et al., 2005). Thus, the regulation of intracellular calcium homeostasis mainly depends on the Ca^{2+}-ATPase pumps found in lens epithelial cells. PMCA has four isoforms (i.e., PMCA1-4), and is a high affinity Ca^{2+} pump. PMCA can use the energy from ATP hydrolysis to drive Ca^{2+} out of the cell against its electrochemical gradient (Brini, 2009). Therefore, PMCA plays an important role involved in the regulation of intracellular calcium homeostasis.

It has been proved that zinc oxide (ZnO) nanoparticles could exert cytotoxic effect on both broncho-alveolar lavage cells and white blood cells in rats *via* interfering with zinc ion homeostasis (Kao et al., 2012), liver cells (Sharma et al., 2011, 2012), and human bronchial epithelial cells (Heng et al., 2010). Our previous studies have also revealed that ZnO nanoparticles can induce rat retinal ganglion cell death *via* reactive oxygen species pathway and Caspase pathways (Guo et al., 2013a). In addition, ZnO nanoparticles could induce apoptosis in human dermal fibroblasts *via* p53 and p38 pathways (Meyer et al., 2011). Recent researches have proposed that nanoparticles can modulate intracellular calcium levels and may play a role in calcium homeostasis (Young et al., 2009a,b; Guo et al., 2013b). This ability which regulates calcium homeostasis is believed to have important physiologic and pathologic implications. However, it is still unknown for the effect of ZnO nanoparticles on human lens epithelial cells (HLECs). In the meantime, the possible mechanism involved in the regulation of calcium homeostasis *via* PMCA has also not been addressed. Thus, in the present study, we explored the cytotoxic effects of ZnO nanoparticles on HLECs in the presence and absence of UVB irradiation through cell viability assay, 4′,6-diamidino-2-phenylindole (DAPI) staining, hydroxyl radical assay kits, flow cytometry, quantitative real-time PCR and western blotting analysis, respectively. Meanwhile, the real-time cell electronic sensing (RT-CES) assay was also applied to explore the dynamic process and binding behavior between cells and ZnO nanoparticles with or without UVB irradiation. We found that ZnO nanoparticles could inhibit the proliferation of HLECs, decrease PMCA1 expression at gene and protein levels, increase the intracellular calcium ion level and further disrupt the intracellular calcium homeostasis, finally cause cell death. These results suggest that cytotoxic effect of ZnO nanoparticles under UVB irradiation on HLEC B-3 cells may be involved in calcium-mediated signaling pathway.

2. Materials and methods

2.1. ZnO nanoparticles

The ZnO nanoparticles (>99.0% purity) capped with aminopolysiloxane were purchased from Jiangsu Changtai Nanometer Material Co., Ltd. and were characterized by a scanning electron microscope (ZEISS EVO, Germany). The particle size distribution and zeta potential of ZnO nanoparticles dissolved in 1640 medium were determined using a Malvern Zetasizer (Malvern Instruments, Britain) with specialized software (Zetasizer Nano ZS). For every experiment, ZnO nanoparticle suspensions were mixed vigorously, sonicated for 20 min on the ice prior to experiment, and then immediately applied to the related assay to minimize agglomeration.

2.2. UV irradiation

For UV irradiation, UVB (λ = 310 nm) was provided by a UVB lamp (Nanjing Huaqiang Electronic Co., Ltd., China). The UVB dose was determined by a double channels UVB illuminometer (Photoelectric Instrument Factory of Beijing Normal University, China). The total exposure dose of UVB was 40 mJ/cm^2 and the average intensity was 0.2 mW/cm^2 at the working plane. In the present study, the effect of different concentrations of ZnO nanoparticles on HLECs was investigated under UVB irradiation for 200 s.

2.3. Cell culture and preparation of ZnO nanoparticle solution

A human lens epithelial cell line (HLEC B-3, purchased from ATCC) was maintained in RPMI-1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (Sigma, USA), 100 U/mL penicillin (Sigma, USA) and 100 μg/mL streptomycin (Sigma, USA) and grown at 37 °C in a 5% CO_2 humidified environment. Cell numbers were determined using an automated cell counter (TC10, Bio-rad, USA). For the preparation of ZnO nanoparticle suspensions, ZnO nanoparticles were dissolved in RPMI-1640 and were sonicated on the ice for 20 min prior to use.

2.4. In vitro RT-CES cytotoxicity assay for HLECs

The RT-CES assay was performed using a RT-CES analyzer (ACEA Biosciences (Hangzhou) Inc., China) according to the literature (Guo et al., 2013b). Initially, 1×10^4 cells were seeded into each well containing 200 μL cell culture medium used for the 16× sensor device and incubated overnight, then the medium was replaced with ZnO nanoparticle suspensions and treated with or without UVB irradiation, followed by incubation at 37 °C for at least 72 h in an incubator contained 5% CO_2. Controls were cultivated under the same condition either without UVB radiation or without ZnO nanoparticles. The relevant experiments were repeated three times independently.

2.5. Viability assay of cells treated with ZnO nanoparticles in the presence and absence of UVB irradiation

The HLECs were seeded on the coverslip in 6-well plate (10^5 cells/well), then treated with different concentrations (i.e., 0, 2.5, 5.0, 10 μg/mL) of ZnO nanoparticles in the presence and absence of UVB irradiation, further all cells were cultured in an incubator with 5% CO_2 at 37 °C for 24 h for optical microscopy assay. After treatment, the cells were observed by an optical microscope (Olympus IX51, Japan). Every experiment was repeated at least three times independently.

2.6. DAPI staining

To monitor the effect of ZnO nanoparticles on HLECs nuclei, the DAPI nuclear staining was carried out. The HLECs were seeded in a 6-well plate at a density of 5×10^4 cells per well and grown overnight, then incubated with different concentrations (i.e., 0, 2.5, 5.0, 10 μg/mL) in 1.5 mL volume of ZnO nanoparticles in the presence and absence of UVB irradiation and further cultured for additional 6 h, subsequently cells were washed with phosphate buffered saline (PBS, pH 7.4). After fixation in 4% polymerisatum for 15 min, the fixed cells were washed with PBS and were stained with 1 μg/mL of DAPI solution (Sigma, USA) for 30 min, then stained cells were examined using an inverted fluorescence microscope (Olympus IX71, Japan) and the typical photographs were captured.