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Zinc oxide nanoparticles induce renal toxicity through reactive oxygen species

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ABSTRACT

Nanoparticles of zinc oxide (ZnO NPs) are applied in many fields nowadays. Consequently, concerns have been raised about its potential harmful effects. The present study focuses on its toxic effect on podocytes and rats. In vitro study, podocytes were treated with different concentrations of ZnO NPs (10, 50 and 100 μg/ml), the viability of cells was decreased as time prolonged according to MTT assay. Meantime, flow cytometry analysis indicated that ZnO NPs induced intracellular accumulation of reactive oxygen species (ROS) and apoptosis. The measurement of superoxide dismutase (SOD) and malondialdehyde (MDA) showed that ZnO NPs decreased SOD level and increased MDA level. Interestingly, pretreatment with N-mercaptopropionyl-glycine, known as a type of ROS scavenger, could inhibit podocyte apoptosis induced by ZnO NPs. Meantime, a loss of nephrin can be detected, which may result in a direct damage to slit diaphragms. In vivo study, adult male Wistar rats were administrated with 3mg/kg/day ZnO NPs for 5 days, body weight and kidney index were significantly reduced. In addition, ZnO NPs decreased the activity of catalase and SOD in kidney cortex in vivo. It could be concluded that ZnO NPs present toxic effect on podocytes and Wistar rats, which was related with oxidative stress.

1. Introduction

The 21st century is regarded by many people as “nanocentury” with the nano-revolution touching almost every aspect of human life such as medical science, drug applications, and defense industries (Long and Ye, 2007). As the nanotechnology develops rapidly, public exposure to nanoparticles is undoubtedly increasing. As a result, the widely application of nanomaterials led to a growing concern on the bioavailability and toxicity of nano-sized materials (Brumfiel, 2003; Igarashi, 2008; Nel et al., 2006; Oberdorster et al., 2005; Nielsen and Lintzeris, 2008).

Although numerous studies on the safety/toxicity of nanoparticles have been published, details have been lacking, however, on the precise mechanisms of injury induced by ZnO NPs. In the present study, the cytotoxic effect of ZnO NPs on podocytes arouses our interest. Nanostructures of ZnO have great potential applications in dyes, paints, textiles, medical diagnosis, sunscreens and cosmetics (Cai, 1995; Mashaghi et al., 2013; Yuranova and Kiwi, 2007). Despite that it is widely used, there are several studies reporting the toxicity of ZnO NPs in bacterial systems, eco-relevant species (Adams et al., 2006; Franklin et al., 2007; Zhu et al., 2008) and vertebrates (Brayner et al., 2006; Heinlaan et al., 2008; Huang et al., 2008). It has been discovered that environmental exposure to ZnO NPs would cause it to stay in the stratum corneum and to accumulate in skin folds and/or hair follicle (Zvyagin et al., 2008). Similarly, the bone, kidney and pancreas were the target organs of 20-nM and 120-nM ZnO (Banyal et al., 2013). On the other hand, exposure to low concentrations of ZnO NPs shows a genotoxic potential mediated by lipid peroxidation and oxidative stress (Sharma et al., 2009). At the same time, several studies reported its damage to a variety of cells such as epidermal cells (Sharma et al., 2009), cancer cells (Akhtar et al., 2012) and liver cells (Sharma et al., 2012). However, there's no related article concerning the toxic effect of ZnO NPs on mouse podocyte cell line.

Podocytes are highly specialized cells, which form multiple interdigitating foot (Pavenstadt et al., 2003). They are

Abbreviations: ZnO NPs, nanoparticles of zinc oxide; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ROS, reactive oxygen species; SOD, superoxide dismutase; MDA, malondialdehyde; N-MPG, N-(mercaptopropionyl)-glycine; SD, slit diaphragm; GFB, glomerular filtration barrier.

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interconnected by slit diaphragms (SD) and cover the exterior basement membrane surface of the glomerular capillary(Pavenstadt et al., 2003). Podocytes play critical roles in the maintenance of glomerular filtration barrier (GFB) (Kretzler et al., 1994). In other words, they would take part in the filtration process, which means the harmful substance, for example, ZnO NPs may accumulate around them. Furthermore, kidney is one of targets for ZnO NPs to retain, which may further promote the accumulation of ZnO NPs around podocytes. As a result, it is necessary to evaluate the influence of ZnO NPs on podocytes and to discover its possible mechanism. At the same time, we became interest in the potential impact of ZnO NPs on the necessary protein of podocytes. To date, there have been limited studies relevant to this topic.

With the establishment of mouse podocyte cell line, which is conditionally immortalized, the study of fully differentiated podocytes in culture has become feasible (Mundel and Reiser, 1997). As a result, the purpose of the study was to assess whether ZnO NPs can induce the apoptosis in podocytes and its impact on a predominate protein, thereby providing basic information for the safe application of ZnO NPs in the future.

2. Materials and methods

2.1. Experimental plan

A brief experimental plan including the selected concentrations and times of the whole study was designed keeping in view the approach for nanomaterials toxicity study as suggested in Vyom Sharma et al. (Sharma et al., 2009), Shichang Liu et al. (Liu et al., 2009) and Jing Xu et al. (Xu et al., 2012)'s report.

2.2. Materials

Rabbit polyclonal anti-nephrin primary antibody was purchased form Abcam, Cambridge, MA, USA. RPMI 1640 culture medium was purchased from GIBCO Invitrogen. The fetal bovine serum (FBS), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma Chemical Co., St Louis, MO, USA. Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit was from Bipec Biopharma Corporation, USA. Reactive oxygen species (ROS) testing kit was purchased from Genmed Scientifics Inc, USA. Plastic culture microplates and flasks used in the experiment were supplied by Corning Incorporated (Costar, Corning, NY, USA). Superoxide dismutase (SOD) assay kit and malondialdehyde (MDA) assay kit were purchased from the Nanking Jiancheng bio-engineering research institute (Nanking, China).

2.3. The characterization of ZnO NPs

The ZnO NPs used in this research was a kind gift from Dr. GG Ren, University of Hertfordshire, England. The characterization of ZnO NPs, such as the size and surface area has been described in our previous work (Zhao et al., 2009).

2.4. Cell culture and preparation of ZnO NPs

Mouse podocytes used in the present study were established by Mundel and Reiser (1997). They were adherent and from a conditionally immortalized cell line. Cells were maintained on plastic culture microplates with RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin and 100 U/ml streptomycin in the presence of 10 U/ml recombinant mouse interferon-γ (IFN-γ) at 33 °C in a humidified atmosphere of 5% CO_2. In order to induce differentiation, podocytes were cultured at 37 °C without IFN-γ (non-permissive conditions) for two weeks.

The stock solution (10 mg/ml) of ZnO NPs was prepared in Milli-Q water and dispersed by the ultrasonic vibration (Branson Inc., Dabury, CT, USA) for 20 min to prevent from aggregation. Then different concentrations of ZnO NPs were prepared from the suspension.

2.5. Animals

Adult male Wistar rats (200 ± 20 g) were purchased from the Laboratory animal center, Academy of military medical science of people's liberation army. All animal experiments were reviewed and approved by the Animal Research Ethics Committee, School of Medicine, Nankai University.

2.6. Induction of acute toxicity on rats

Adult male Wistar rats were randomly assigned into two groups, i.e. control group (n = 8) and ZnO NPs group (n = 8)). Rats were administrated with 3 mg/kg/day by intraperitoneal injection. At the end of 5 days of administration, rats were killed and kidney cortex were collected and stocked at −70 °C.

2.7. Cell viability assay

The MTT assay was used to assessed the cell viability (Denizot and Lang, 1986). In brief, the cells (1×10^4/ml) were seeded into each well in 96-well culture plates and incubated with various concentrations of ZnO NPs (10 μg/ml, 50 μg/ml and 100 μg/ml) for the periods of 3 h, 6 h, 12 h and 24 h. 20 μl MTT was added to each well after the exposure, at a final concentration of 0.5 mg/ml and incubated for another 4 h at 37 °C. The medium was then removed carefully. 150 μl DMSO was added in after that and mixed with the cells thoroughly until formazan crystals were dissolved completely. An ELISA reader (Elx 800, Bio-TEK, USA) was used to measure the mixture at 570 nm. The cell viability was expressed as a percentage of the control. Meanwhile, the concentrations of the ZnO NPs used in assays of SOD and MDA were based on the results of the MTT test.

Cell viability (%) = (the viability of ZnO NPs treated group/the control group) × 100.

2.8. Detection of apoptotic cells by flow cytometry

In order to determine the effect of ZnO NPs on podocyte apoptosis, the apoptosis was assayed by annexin V-FITC and PI staining followed by analysis with flow cytometry (BECKMAN-COULTER, USA). The methodology followed the procedures as described in the annexin V-FITC/PI detection kit. In brief, after treatment with different concentrations of ZnO NPs (10 μg/ml, 50 μg/ml and 100 μg/ml) for 12 h, cells were harvested and washed twice with pre-cold phosphate buffer saline (PBS) and resuspended in 1 × binding buffer with a concentration of 1×10^6 cells/ml. The cells were stained with 5 μl annexin V-FITC and 5 μl PI for 15 min at room temperature after that in the dark. Then the cells were analyzed by flow cytometry.

2.9. Measurement of ROS

The generation of ROS was measured using intracellular oxidation of dichlorodihydrofluorescein diacetate (DCFH-DA) at 37 °C for 20 min in the dark. The cells were pre-loaded in a 6-well plate for 24 h for stabilization. Then cells were washed and incubated with different concentrations of ZnO NPs (10 μg/ml, 50 μg/ml and 100 μg/ml) for 6 h. After exposure, cells were washed with PBS. Then they were resuspended at a concentration of 1×10^6 cells/ml.