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The Effects of Different Sizes of Nanometer Zinc Oxide on the Proliferation and Cell Integrity of Mice Duodenum-Epithelial Cells in Primary Culture

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Abstract: The MTT activity and LDH release were investigated in the proliferation and cell integrity of mice duodenum-epithelial cells which exposed to 10-15 nm and 100 nm nanometer zinc oxide (nano-ZnO). The mice duodenum-epithelial cells supplied two different sizes of nano-ZnO where supplemental Zn concentrations were 0, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 μg ml^{-1}, respectively. The results showed that the MTT activity increased with the increased concentrations of 10-15 nm and 100 nm nano-ZnO, while the concentration 12.8 μg ml^{-1} of 10-15 nm nano-ZnO decreased the MTT activity. The LDH released increased significantly under the concentration 12.8 μg ml^{-1} of 100 nm nano-ZnO and 6.4 μg ml^{-1}, 12.8 μg ml^{-1} of 10-15 nm nano-ZnO. In summary, the nano-ZnO promoted the proliferation of the cells and did not injury the cells at lower concentrations.

Key words: Duodenum-epithelial cell, nano-ZnO, proliferation, integrity

INTRODUCTION

Dietary levels of 2,000-4,000 ppm of Zn from ZnO can enhance animal growth and it has been generally utilizing in current animal product (Case and Carlson, 2002). However, the high levels of Zn excreted by supplemented have raised concerns about its potential environmental pollution (Buff *et al*., 2005). The Emerging of nanomaterials and nanotechnology may lead the new way for solving the contrary. Nanomaterials and nanotechnology began to emerge some 20 years ago. The material which dimensions below 100 nm is called nanomaterials. It has many functions, such as quantum dimension effect, minor dimension effect, surface effect and quantum tunnel (Behrens *et al*., 2002). Because of its small size, it is easier to uptake by the gastrointestinal tract. It has great interests whether supplemented less nanometer zinc oxide (nano-ZnO) can achieve the similar effect of ZnO.

The duodenum-epithelial cell is very important for host to digest and uptake nutrition in gut. It has great contribution to body health and growth. The epithelium cells *in vitro* provide an attractive model for the study of epithebiab proliferation and differentiation (Gordon, 1989). If properly validated, the advantages of these early screening tests would result since they are simpler, faster and less expensive than their *in vivo* counterparts. It is profit to evaluate new particulate materials in small quantities.

It has been proved by previous research *in vitro* that the exorbitant levels of nanomaterials can led to cell toxicity (Hsin *et al*., 2008; Jeng and Swanson, 2006). Nanomaterials adverse effects were partly due to the result of the Reactive Oxygen Species (ROS) (Yamamoto *et al*., 2004), or causing membrane damage (Reddy *et al*., 2007) and so on. However, there was less study about relatively lower concentration of nano-ZnO on intestine epithelial cell. The research found that Hela cell line showed a good reproduction behavior at lower nano-ZnO concentration. (Li *et al*., 2008). For supplying the proof of using nano-ZnO in animal, we decide using mice duodenum -epithelial cells for model, investing the effect of different levels of 10-15 and 100 nm nanometer zinc oxide (nano-ZnO) on the proliferation and cell integrity.

MATERIALS AND METHODS

Particles preparation: The culture media supplemented 2 μmol ml^{-1} *N*, *N*, *N'*, *N'*-tetrakis (2-pyridylmethyl) ethylenediamine (TEPN) to chelation the Zn^{2+} of the media. Each particle were prepared in ultrapure water, Phosphate-buffered Saline (PBS) solution (sterile filtered) and DMEM cell culture media. Each suspension was probe sonicated for 30 min prior to exposure and characterization (Jiang *et al*., 2009). For the in vitro studies, each stock solution was diluted serially to yield seven concentrations ranging from 0 μg ml^{-1} to 12.8 μg ml^{-1}. Then, the culture media was added to plates immediately.

Epithelial cell culture: Male and female twelve -day-old mice were used. The animals were killed by cervical dislocation and the 1 cm length of the duodenum was removed (cleaned free of mesentery). The method of cell isolation for mice intestinal epithelial cell was using conditions described previously (Mariadason *et al*., 2005; Evans *et al*., 1992). Cells were seeded in 96-well

plates and were grown in DMEM supplemented with 2 mM L^{-1} glucose, 100,000 U L^{-1} penicillin, 100,000 μg L^{-1} streptomycin sulfate and 5% fetal bovine serum and maintained at 37°C in an atmosphere of 5% CO_2, 95% air at 90% relative humidity. The medium was changed every 48 h and the cells were left for 7-10 d to allow differentiation.

MTT activity: Cells were seeded in 24-well plates and exposed to increasing concentrations of particle suspensions (0, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 μg ml^{-1}). After 24 h incubation, the cell culture media were aspirated; 400 μl of MTT (5 mg ml^{-1}) was added to each well and incubated for 4 h. Afterwards, the MTT were aspirated; 400 μl of dimethyl sulfoxide (DMSO) solubilization solution was added to each well. The resulting formazan crystals were solubilized in DMSO and quantified by measuring absorbance at 570 nm (Fukamachi, 1992).

LDH release: Cells were seeded in 96-well plates, exposed to particle suspensions as mentioned before. After 24 h incubation, the cell culture media were aspirated. The media were transferred into the fresh centrifuge tubes and analyzed for LDH release using LDH kits which were purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China. Data were collected and recorded by measuring absorbance at 440 nm (He *et al*., 2008).

Statistical analysis: The results obtained were analyzed statistically using one factorial analysis of variance with least squares method with the analysis program SPSS. Data were tested at significance levels of $p<0.05$.

RESULTS

Effect of nano-ZnO on MTT activity: The MTT activity increased with the increased concentrations of 100 nm nano-ZnO. It was significant increased in levels at 1.6, 3.2, 6.4, 12.8 μg ml^{-1} ($p<0.05$) and reached the highest numerus at 12.8 μg ml^{-1}. While 10 nm nano-ZnO produced significant increased in MTT activity at 0.8, 1.6, 3.2, 6.4 μg ml^{-1} ($p<0.05$) and reached the highest numerus at 6.4 μg ml^{-1}. MTT activity decreased significantly when concentration reached 12.8 μg ml^{-1} (Fig. 1).

Effect of nano-ZnO on LDH release: The LDH release did not increase with the increased concentrations of nano-ZnO. 100 nm nano-ZnO produced significant increased in LDH release at 12.8 μg ml^{-1} ($p<0.05$). While 10 nm nano-ZnO produced significant increased in LDH release at 6.4, 12.8 μg ml^{-1} ($p<0.05$) (Fig. 2).

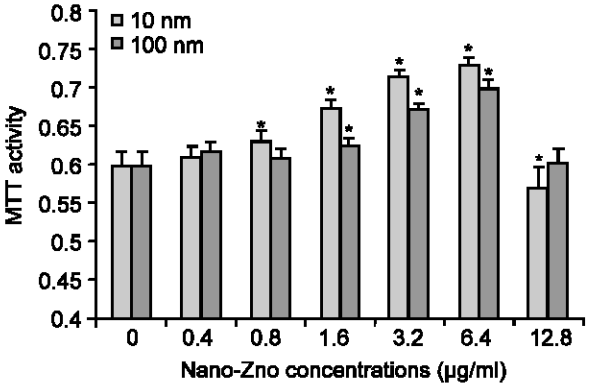

Fig 1: Effect of nano-ZnO on MTT activity *in vitro* culture (*p<0.05)

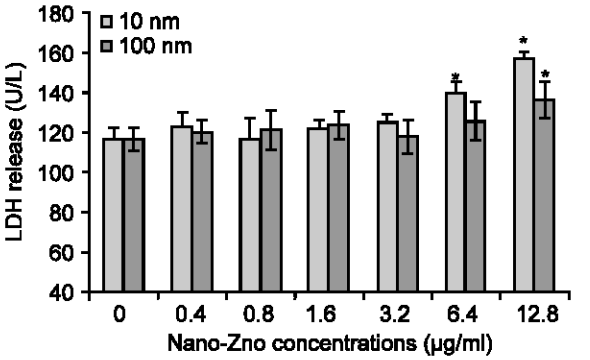

Fig 2: Effect of nano-ZnO on LDH release *in vitro* culture (*p<0.05)

DISCUSSION

The duodenum is the interface between the external and internal environments. The proliferation and cell integrity of epithelial cells are very important to host (Platt and Mowat, 2008). Previous research has found that ZnO can enhance the growth and reparation of epithelial cells (Lansdown, 1995). The proliferation of intestinal epithelial cells in culture was quantitatively measured by MTT assay in this study. The results showed that nano-ZnO encouraged the proliferation of the cells. The 3.2 μg ml^{-1} 10-15 nm nano-ZnO has the similar effect with 6.4 μg ml^{-1} 100 nm ZnO.

It has been proved in mice that 100 nm particles have 10-250 times absorptivity than bigger particles (Desai, 1992). Because of the degree of uptake in gut has the great relationship with the interfacial area and dissolution speed in absorption region (Florence, 1998), the smaller particle is the better to uptake by gut. Therefore, 10-15 nm particles probably enter the cells directly and then, enhance the cell growth by influence some relative zinc finger protein or zinc enzyme levels (MacDonald, 2000).

Otherwise, nano-ZnO has the double characteristic both ZnO and nanomaterials. And excessive naro particles are harmful to cells (Igarashi, 2008). Naroparticles will react with maters in cells after entering. Nano-ZnO will enhance cell growth in less concentration, while excessive naro particles can devitalize cell protein and

damage DNA (Vyom Sharma *et al*., 2009). LDH release can reflect cell integrity, because LDH will increased when cellular membrane broken. Under our experimental condition, we found that 100 nm nano-ZnO will enhance the LDH release at higher concentration and 10 nm nano-ZnO did not affect the LDH release at lower concentration. Because our trials were under lower levels of nano-ZnO, the result was different from previous study. So, it was obviously that epithelial cell grown well without cellular membrane broken under lower levels of nano-ZnO from the trial. It was imply that nano-ZnO has minor dimension effect and dosage effect to cells. On the whole, 10-15 nm nano-ZnO enhanced cell growth efficiency than 100 nm nano-ZnO at lower concentrations and did not injury the cells. In addition, more research is needed to designate the function changes of cells under lower levels of nano-ZnO. And the smaller sizes of nano-ZnO need to be further study *in vitro*.

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