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Protective Effects of Nano-ZnO on the Primary Culture Mice Intestinal Epithelial Cells in *in vitro* Against Oxidative Injury

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Abstract: Nano-ZnO is a new products, which may used to be feed resources. The aim of this study was to exam, whether nano-ZnO is able to protect cell integrity from potential damage by free-radical oxidative injury. A 3×3 factorial experiment was used to investigate the interaction between particle diameter (15, 50 and 100 nm) and concentration (0.4, 1.6 and 6.4 μg mL^{-1}) of the primary culture mice Intestinal Epithelium Cell (IEC) in *in vitro*. The results indicated that the all the nano-ZnO groups MTT OD value were significantly higher than the perhydrol group ($p<0.05$) and LDH decreased, but not significant ($p>0.05$). The MDA content in all the nano-ZnO groups were decreased significant ($p<0.01$). The SOD and CAT in all nano-ZnO groups were higher than the perhydrol group ($p>0.05$). The results shown that nano-ZnO released the IEC oxidative injury.

Key words: Intestinal epithelium cell, nano-ZnO, antioxidant, antioxidant enzymes, integrity, *in vitro*

INTRODUCTION

The role of zinc in the protective effect in resisting intestinal disease is well known (Schell and Kornegay, 1996; Shankar and Prasad, 1998). In particular, several studies conducted on piglets show that pharmacological concentrations of zinc (from ZnO) prevent or alleviate diarrhea, which is mainly caused by oxidative stress (Powell, 2000; Hahn and Baker, 1993). Zinc plays an important role in maintaining membrane function and stability. Some researchers suggest that the mechanism of this activity involves the stabilization of the membrane structure (O'Dell, 2000) or the displacement of redox-active metals to prevent free-radical oxidative damage (Canali *et al*., 2000). Nano-ZnO is a new product whose particle diameter is between 1-100 nm. Recently, nano-ZnO attracted much attention gradually in the area of animal science. Its strong energy restriction effect and interface effect, which lead to a higher electron conductivity, transparency and transmission ability than the bulk ones and other metallic oxide materials (Joshy and Abdul, 2001), will open more opportunities to make use of this nano-material.

The integrity of the intestinal barrier is fundamental to the proper functioning of the epithelial cells (Lu and Walker, 2001). In many published studies, zinc plays a role in maintaining epithelial barrier integrity and function (Bao and Knoell, 2006; Roselli *et al*., 2003). Whether, nano-ZnO is able to protect membrane integrity from potential damage by free-radical oxidative injury remains to be elucidated. Therefore, to understand the effect of anti-oxidative activity of nano-ZnO, we designed to examine the primary culture mice Intestinal Epithelium Cell (IEC), the effects of different diameter and levels on IEC survival rate. Moreover, we determined the L-lactate Dehydrogenase (LDH), Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Catalase (CAT) in response to the different treatments.

MATERIALS AND METHODS

Experimental animals: Seven days-old mice were obtained from the National Experimental Teaching Demonstration Center of Animal Science, Ya'an, China. All animal protocols were approved by the Institutional Animal Care and Use Committee of Sichuan University of Agricultural (Ya'an, China).

Experimental design: The study used a 3×3 test design that tested f or 3 factors: the particle diameter 15 nm nano-ZnO group (1-1, 1-2 and 1-3 group), the 50 nm nano-ZnO group (2-1, 2-2 and 2-3 group) and the 100 nm nano-ZnO group (3-1, 3-2 and 3-3 group). The 3 levels were 0.4, 1.6 and 6.4 μg mL^{-1}, respectively. In addition, there were a Perhydrol Group (PG) and a Normal Group (NG). For each level of 8 to repeat and repeated each of 1 well.

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Cell culture and the determination of indicators: The method of cell isolation for mice Intestinal Epithelial Cell (IEC) was according to Evans *et al.* (1992). Cells were diluted to 1×10^5 mL^{-1} and seeded in 96 and 24 well plates with 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 placed into an incubator with 37°C, 5% CO_2 (BB5060UV type, Heraeus, Germany). The medium was changed every 48 h and the cells were left for 7-10 days to allow differentiation and then different concentrations of 3 particle diameter nano-ZnO (final concentration were 0.4, 1.6 and 6.4 μg mL^{-1}, respectively) were added for 4 h to which, H_2O_2 (final concentration of 200 μM L^{-1}) was added except the normal group and placed into an incubator with 37°C, 5% CO_2 for 24 h.

After the incubation, each well of the 96-well plates by adding Thiazolyl blue (MTT) solution (5 g L^{-1}) 10 μL, continued to train for 4 h. Centrifuge to the supernatant, adding dimethyl sulfoxide 100 μL and was fully shocked after 20 min. The calorimetric detection of the optical density value at 570 nm wavelength was analyzed using a fully automatic micro plate reader (Wellsan MK type, Thermo, USA).

The collection of cells and culture solution in every 24 well plates well in 1.5 mL centrifuge tubes, centrifuged at 2000 r min^{-1} for 20 min, the collection of the supernatant and sediment saved at -20°C for the test LDH, MDA, SOD and CAT assays. All the test using kits, which were purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

Statistical analysis: All data were expressed as mean±SD. A one-way Analysis of Variance (ANOVA) was used to perform comparisons using SPSS (version 14.0; SPSS Inc., Chicago, III). Group differences resulting in $p<0.05$ (0.01) were considered to be statistically significant (very significant).

RESULTS AND DISCUSSION

The influence of different particle diameter and level of nano-ZnO on cell MTT OD value is shown on Fig. 1a. All the nano-ZnO groups OD value were significantly higher than the perhydrol group ($p<0.05$). The 15 nm, 0.4 and 1.6 μg mL^{-1}groups OD value were very significantly higher than the other nano-ZnO groups and the differences among the 15 nm, 0.4, 1.6 μg mL^{-1} and NG groups was not significant ($p>0.05$).

LDH, MDA, SOD and CAT content are shown in Fig. 1b-e. The addition of nano-ZnO decreased LDH, but not significant ($p>0.05$). The MDA content in all the nano-ZnO groups were decreased significant ($p<0.01$), compared to the perhydrol group and the 15 nm nano-ZnO group seemed lower than any other diameter group. As for the antioxidase activities, SOD and CAT. All the nano-ZnO groups were higher than the perhydrol group. The trend of influence of the nano-ZnO groups on SOD activities was in an downward trend along with the particle diameter augmentation. However, the influence among different particle diameters and levels of nano-ZnO on CAT were not significant ($p>0.05$).

Zn plays an essential role in cell membrane integrity and is a component of >300 different enzymes that function in many aspects of cellular metabolism, involving metabolism of proteins, lipids and carbohydrates (Parkin, 2004; McCall *et al.*, 2000). However, the mechanism of the nano-ZnO protective effect is largely unknown. We hypothesized that nano-ZnO could release the IEC oxidative injury so as to gain a better performance of cells. Previous research has found that ZnO can enhance the reparation of epithelial cells (Lansdown, 1995). In present study, all the nano-ZnO groups OD value were significantly higher than the perhydrol group ($p<0.05$), indicated that nano-ZnO enhanced the number of live cells and it seems that the effect of small diameter better than the effect of large one.

The generation of Reactive Oxygen Species (ROS), which derive from electron transfer reactions in mitochondria and endoplasmic reticulum is inherent in aerobic cell metabolism (Stehbens, 2003). The cell would be injury when exposure a high concentration ROS environment. MDA is the by products of cell membrane lipid peroxidation. In present study, either small- or large-diameter nano-ZnO decrease LDH ($p>0.05$) and MDA ($p<0.01$), compared to that of perhydrol group. The results indicated that nano-ZnO released lipid peroxidation in mitochondria and cell membranes, so that stabilized the cell membrane structure. This is in close agreement with previous studies of Stehbens (2003) and Tapiero and Tew (2003). It was in the expectation that cells supplemented with nano-ZnO had the better performance and higher antioxidant enzymes activities, which may due to the enhance of cell producing more SOD and CAT enzymes. The formations of superoxide and peroxide are inextricably linked, as superoxide is converted to peroxide and oxygen, a reaction catalyzed by SOD. SOD plays a role in modulating ROS and is sensitive to Zn (Stehbens, 2003). The cytotoxic effects of hydrogen peroxide (H_2O_2) are limited by its degradation by CAT. CAT, localized in peroxisome, exerts a function: decomposition of H_2O_2 to yield H_2O and O_2 (Alessia *et al.*, 2007; Santon *et al.*, 2003) have shown that the maintenance on basal levels of the antioxidant enzymes activities support the hypothesis that Zn can retard oxidative mechanisms, suggesting a role in modulating ROS.

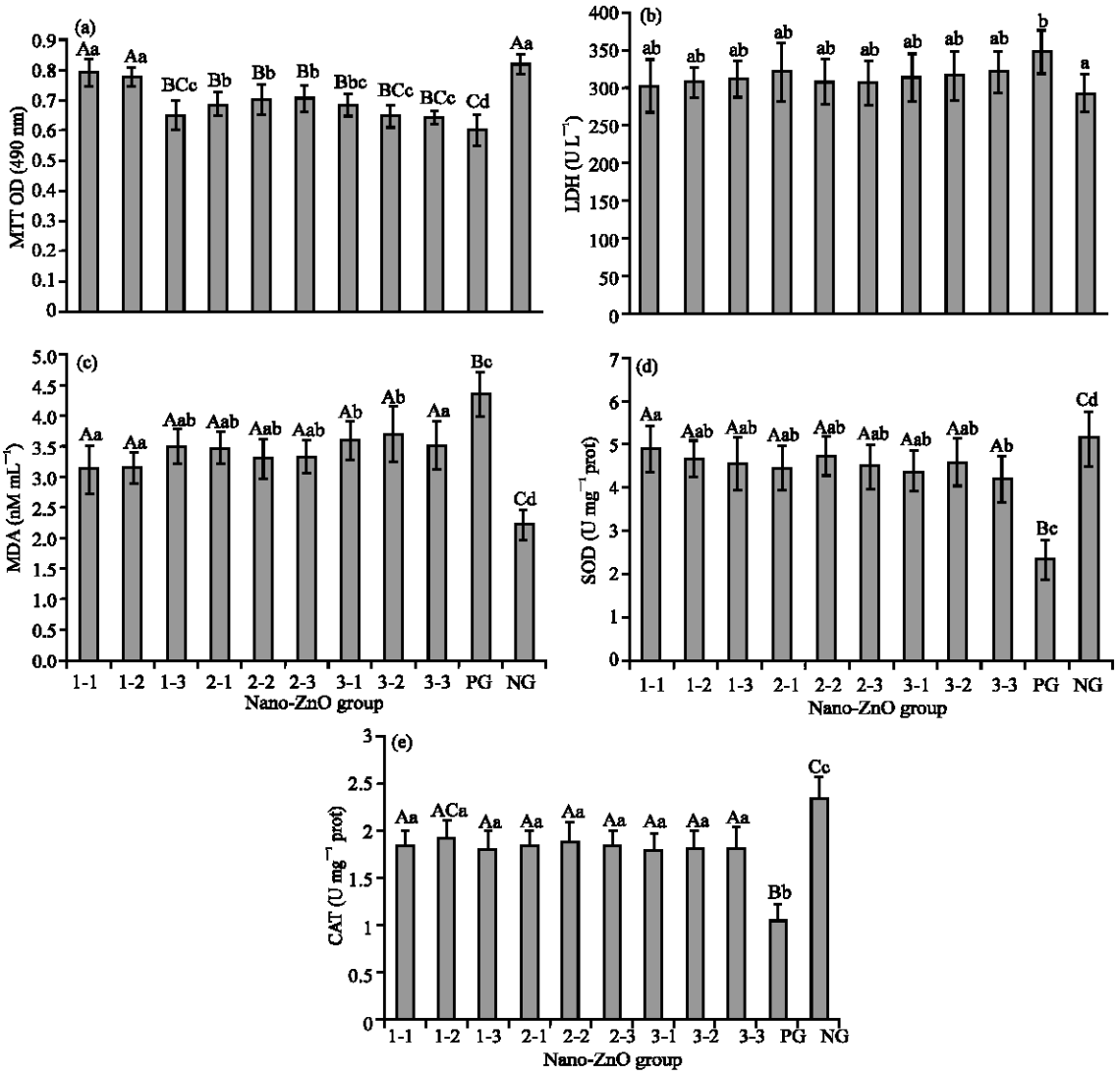

Fig. 1: The influence of nano-ZnO on the a) MTT OD value , b) LDH release, c) MDA release, d) SOD of mice and e) CAT of mice intestinal epithelium cell in *in vitro* culture. The difference between data with different capital letters was significant among groups ($p<0.01$) and the difference between data with different small letters was significant among groups ($p<0.05$) and the same letters was not significant ($p>0.05$)

CONCLUSION

The present study shown that nano-ZnO released the IEC oxidative injury and enhance SOD and CAT enzymes activities. However, further studies are needed in order to find the mechanism of the nano-ZnO protective effect.

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