J Nanopart Res (2008) 10:263–276
DOI 10.1007/s11051-007-9245-3

RESEARCH PAPER

Acute toxicological impact of nano- and submicro-scaled zinc oxide powder on healthy adult mice

Bing Wang · Weiyue Feng · Meng Wang · Tiancheng Wang · Yiqun Gu · Motao Zhu · Hong Ouyang · Junwen Shi · Fang Zhang · Yuliang Zhao · Zhifang Chai · Haifang Wang · Jing Wang

Received: 8 October 2006 / Accepted: 13 April 2007 / Published online: 20 June 2007

Abstract In this work, the acute oral toxicity of 20- and 120-nm ZnO powder at doses of 1-, 2-, 3-, 4-, 5-g/kg body weight was evaluated referred to the OECD guidelines for testing of chemicals. As the results, both 20- and 120-nm ZnO belong to non-toxic chemicals according to the Globally Harmonized Classification System (GHS) for the classification of chemicals. The distribution determination showed that Zn was mainly retained in the bone, kidney and pancreas after 20- and 120-nm ZnO administration. However, the results of blood measurement suggest that the increase in blood viscosity could be induced by low and median dose of 20-nm ZnO but high dose of 120-nm ZnO. The pathological examination showed that the 120-nm ZnO treated mice had dose–effect pathological damages in stomach, liver, heart and spleen, whereas, 20-nm ZnO displayed negative dose–effect damages in liver, spleen and pancreas. Therefore, we conclude that the liver, spleen, heart, pancreas and bone are the target organs for 20- and 120-nm ZnO oral exposure. More attention should be paid on the potential toxicity induced by low dose of 20-nm ZnO oral exposure.

Keywords Acute oral toxicity · Nano-meter zinc oxide powder · Submicro-meter zinc oxide powder · Mice · Toxicology · Health effects · Medicine

B. Wang · W. Feng (✉) · M. Wang · M. Zhu · H. Ouyang · J. Shi · Y. Zhao · Z. Chai
Laboratory for Bio-Environmental Health Sciences of Nanoscale Materials and Nanosafety and Key Laboratory of Nuclear Analytical Techniques, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China
e-mail: fengwy@mail.ihep.ac.cn

B. Wang · M. Wang · M. Zhu · J. Shi · F. Zhang
Graduate School of Chinese Academy of Sciences, Beijing 100049, China

T. Wang
Department of Clinical Laboratory of Medicine, 3rd Hospital of Peking University, Beijing 100083, China

Y. Gu
Maternity Hospital of Haidian District, Beijing 100080, China

Z. Chai
Institute of Nanochemistry and Nanobiology, Shanghai University, Shanghai 200444, China

Z. Chai
Institute of Nuclear Technology, Shenzhen University, Shenzhen 518060, China

H. Wang · J. Wang
College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China

Introduction

In the last decade, several epidemiological studies have found high associations of ambient ultrafine particles (d < 100 nm) with respiratory and

cardiovascular diseases of humans (Pekkanen et al. 1997; Penttinen et al. 2001; Peters et al. 1997a, b; von Klot et al. 2002; Wichmann et al. 2002). It has demonstrated that nanoparticles, such as titanium oxide, carbon, and carbon nanotubes, may cause more inflammation than larger particles of the same materials at a same mass dose delivery (Rahman et al. 2002; Oberdörster et al. 2000; Lam et al. 2004). Nowadays, in addition to the great amount of nanoparticles released from combustion processes, more and more nanoparticles from nanotechnological industry continue to grow and appear in the air, water, plant, soil and many other environmental mediums. Since new properties usually emerge in nano-scaled particles, it is emergent to know about their typical toxicological and environmental effects via direct and indirect exposure.

In our previous study, we evaluated the acute toxicity of oral exposure to nano-scaled zinc metal powder in mice and found that nano-scaled Zn powder could induce severer anemia and renal damage than micro-scaled Zn (Wang et al. 2006). In this study, we considered the engineered zinc oxide nanoparticles because nano-sized ZnO, as a piezoelectric as well as semiconductive material, has been widely applied in electronics (Banerjee et al. 2002), optoelectronics (Lee et al. 2002), gas sensors (Lee and Lee 2001) and so forth. Nano-sized ZnO can also be used in environmental remediation because of its good absorptive and photocatalytic properties for elimination or degradation of pollutants in water or air (Qiang 2001). Similarly, as TiO_2 nanoparticles, nano-sized ZnO has been also used in sunscreen and cosmetics (The Royal Society & the Royal Academy of Engineering 2004). Though the toxicological evaluation of zinc oxide reported by SCCNFP (Scientific Committee on Cosmetic Products and Non-food products 2003) have been showed that the LD_{50} of normal ZnO for rats is more than 5-g/kg body weight and belongs to non-toxic chemicals demonstrated by a single oral ingestion. However, the final opinion of SCCNFP states that an appropriate safety dossier on micro-sized ZnO including possible pathways of cutaneous penetration and systemic exposure is still required. Considering the unique physicochemical properties, including small size effect, large specific surface area, extremely high biological surface reactivity and so forth, nanoparticles might affect the toxicological behavior of materials in organisms, therefore, it needs to further investigate whether the toxicity of nano-scaled materials is related to their particle size. The investigation of the acute oral toxicity of nanomaterials could provide the basic data of their toxicological evaluation.

In the present work, the acute oral toxicity of nano-scaled ZnO (~20 nm) and submicro-scaled ZnO (~120 nm) powder at different doses were evaluated referred to the OECD guidelines for testing of chemicals (OECD 2001). Additionally, the accumulation of Zn in the organic tissues after oral administration and the effects of the particle exposure on the mouse blood-elements, serum biochemical levels and blood coagulation indexes have been investigated. The histopathological changes have been examined as well.

Materials and methods

Characterization of nanometer and submicrometer ZnO

The 20-nm ZnO powders were purchased from Jiangsu Haitai Nanomaterials Co. Ltd (Jiangsu Province, China) and 120-nm ZnO powders were from Xinyu Huaderun Fine Chemical Plant (Jiangxi Province, China). The size distribution of 20- and 120-nm ZnO in the administration solution (1% sodium carboxy methyl cellulose) was performed using a 90Plus Particle Size Analyzer (PSA) equipped with 50-mW solid state laser operating at 659nm wavelength (Brookhaven Instrument Corp).

The purities of the 20- and 120-nm ZnO particles were analyzed by ICP-AES (Baird ICP2070, USA) determination (see Table 1).

Dissolution of ZnO nanoparticles in artificial gastric fluid

In order to evaluate the dissolution of ZnO nanoparticle in gastric fluid, the dissolution studies of the 20- and 120-nm ZnO particles *in vitro* were carried out in 500 ml artificial gastric fluid (0.2% sodium chloride, 0.32% pepsin, 0.7% hydrochloric acid, pH 1.2) at 37 °C stirring for 10, 15, 25, 35, 45, 60 and 120 min. The 5-ml supernatant was collected and the Zn content was analyzed by inductively coupled plasma

Table 1 Percentage of impurity metals in 20- and 120-nm ZnO particles (%)

Samples	NiO	PbO	Fe_2O_3	SiO_2	CaO	TiO_2	MnO	CuO
20 nm	0.0001	0.0005	0.0006	0.0025	–	0.0005	0.0006	0.004
120 nm	0.001	0.02	0.03	0.029	0.001	0.0003	0.003	0.006

–, Below the detection limit

mass spectrometry (ICP-MS, Thermo Elemental X7, Thermo Electron Co.). The mean of three determinations was used to calculate the dissolution percentage of ZnO nanoparticle in the artificial gastric fluid.

Animals and treatment

Animal experiment was performed with compliance of the local ethics committee. The healthy CD-ICR mice, weight about 20–22 g (8-weeks old) were supplied by the Experimental Animal Center, Peking University. The animals were housed in clean polypropylene cages and maintained in an air-conditioned animal house at 20 ± 2 °C, 50–70% relative humidity and 12-h light/dark cycle. The animals were provided with commercial rat pellet diet and deionized water *ad libitum*. After one week acclimation, the mice were randomly divided into three groups: 20-nm ZnO, 120-nm ZnO and the control. Each group consisted of five female and five male mice.

The ZnO particles were suspended in 1% sodium carboxy methyl cellulose and dispersed by ultrasonic vibration for 15 min. The animals were kept fasting over night before treatment. The mice were administered by 1-, 2-, 3-, 4-, and 5-g/kg body weight 20- and 120-nm ZnO, labeling as N1, N2, N3, N4, N5, and SM1, SM2, SM3, SM4, SM5, respectively, for dose–effect and size-effect studies. The control group was given by 1% sodium carboxy methyl cellulose solution instead. The highest dose of 5-g/kg body weight was selected on the basis of the evaluation of SCCNFP on normal ZnO chemical (SCCNFP 2003), which indicated that the LD_{50} of ZnO was more than 5-g/kg and no death occurred at this dose. After administration, the skin and fur changes, eye secretion, respiration and behavior patterns of the mice were observed. Special attention was paid on the clinical signs of toxicity including tremors, convulsions, salivation, nausea, vomiting, diarrhoea, lethargy, coma, etc. The body weights of mice were recorded before and every two days interval after the administration.

Two weeks later, the animals were sacrificed and the blood was obtained from ophthalmic veins. The heart, liver, spleen, stomach, kidneys, pancreas, testis or uterus and the brain were collected. Two small blocks of the above organic tissues of both male and female mice for each group were kept in 10% formalin for histopathological examination.

Biochemical assay of serum

The serum was obtained by centrifugation of the whole blood at 3000 rpm for 15 min. The serum biochemical levels including lactate dehydrogenase (LDH), alanine aminotransferas (ALT), alkaline phosphatase (ALP), leucine aminopeptidase (LAP), total protein (TP), total cholesterol (TC), tri-glyceride (TG), uric acid (UA), creatinine (CR), serum phosphorus (P), and alpha-hydroxybutyrate dehydrogenase (HBD) were assayed by an automatic biochemical analyzer (7170A, Hitachi, Tokyo).

Blood-element test and blood coagulation examination

The 0.1 mL of 15-g/L EDTA-Na was pre-added into 1 mL whole blood sample and the anticoagulant blood sample was immediately used for the blood-element test within 2 h. The blood-elements, including white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), haematocrit (HCT), mean corpuscular hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red cell distribution width corpuscular volume (RDW-CV) and blood platelet (PLT) were assayed by an automatic hematology analyzer (SYSMEX Co. Ltd kx-21N, Japan).

The 0.1 mL of 3.8% sodium citrate was pre-added into 1 mL whole blood sample and the blood plasma was obtained by centrifugation at 5,000 rpm for 5 min for blood coagulation examination. The blood coagulation related markers, including prothrombin time (PT), activated partial thromboplastin time (APTT)

and fibrinogen (FIB), were determined by enzyme-linked immunoadsordent assay (ELISA).

Zn content analysis

In order to investigate the accumulation of Zn in vivo after 20- and 120-nm ZnO oral exposure, the Zn content in the serum and organic tissues of 5 g/kg treated mice was analyzed. The viscera samples were frozen-dried and predigested in 3 ml ultrapure grade nitric acid overnight, then 0.5-ml H_2O_2 was added and the mixture solution was digested at about 160 °C. The Zn content was analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Elemental X7, Thermo Electron Co.) method.

Histopathological observation

A small piece of heart, liver, kidney, spleen, lung, pancreas, stomach, and brain was fixed by 10% formalin and then embedded into paraffin, sectioned for 5–6-μm thick, and mounted on the glass microscope slides using standard histopathological techniques. The sections were stained with hematoxylin-eosin and examined by light microscopy.

Statistical analysis

The data were expressed as mean ± standard deviation. For statistical analysis, the experimental values were compared to their corresponding control ones. A one-way analysis of variance (ANOVA) in SPSS software (Version 11.0) was used to illustrate the significant difference between the experimental group and the control. The significant difference was considered to be $P < 0.05$.

Results and discussion

Particle properties

The average sizes of 20- and 120-nm ZnO particle were measured as 20 ± 5 and 120 ± 20-nm, respectively, by TEM (Fig. 1). Both the particles are close to spherical or elliptical shape (Fig. 1). The 20- and 120-nm ZnO particles in 1% sodium carboxy methyl cellulose administration solution could form monodisperse system (Fig. 2). The particle sizes of

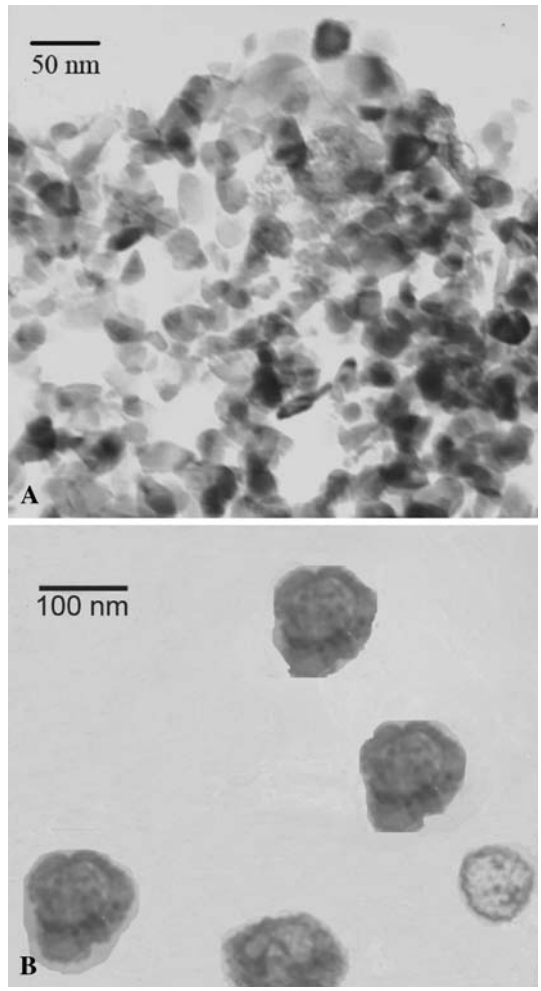

Fig. 1 (A) TEM image of 20-nm ZnO; (B) TEM image of 120-nm ZnO

20- and 120-nm ZnO in the administration solution are about 44.8 ± 16 nm and 187.5 ± 1.3 nm, respectively, which are slightly larger than the results measured by TEM. The specific areas of 20- and 120-nm ZnO are calculated as 4.4×10^5 cm^2/g and 9.1×10^4 cm^2/g, respectively. The particle numbers of 20- and 120-nm ZnO for 1-μg mass weight are 2.2×10^{10} and 2.0×10^8, respectively. The purities of 20- and 120-nm ZnO are higher than 99.5% (See Table 1) that only a small amount of Ca, Cu, Fe, Mn, Ni, Pb, Si and Ti oxide compounds exist, indicating the toxic effects observed in this study are under 20-nm ZnO exposure itself. The 20-nm ZnO exhibits a little higher dissolution rate (0.021%/min) than 120-nm ZnO (0.016%/min) in the artificial gastric

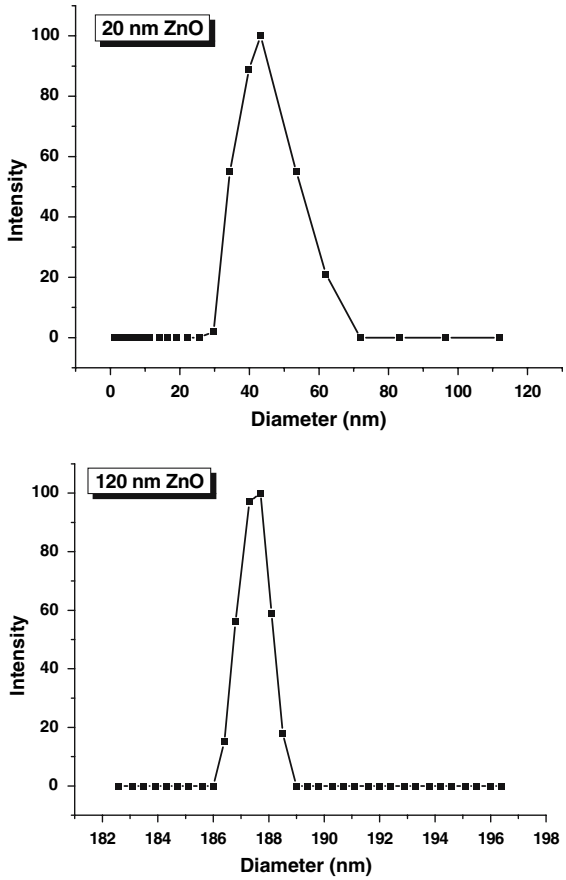

Fig. 2 Size distribution of 20- and 120-nm ZnO in 1% sodium carboxy methyl cellulose solution

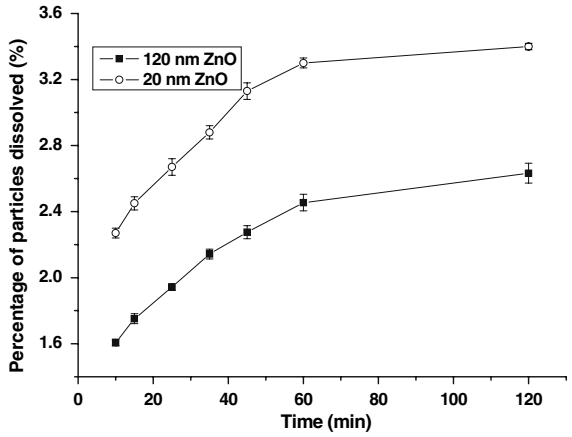

Fig. 3 Dissolution rate of 20- and 120-nm ZnO in the artifical gastric fluid

fluid (AGF) at the first dissolution hour, totally 3.3% and 2.4% dissolution for 20- and 120-nm ZnO, respectively (Fig. 3). But thereafter, the dissolution rate slowed down quickly. Considering the volume of gastric fluid of a mouse is only about 1 ml, in fact only a little amount of ZnO particles could be dissolved.

Acute toxic observation

The numbers of death in each dose group after administration were recorded (Table 2). As the results, the female mice are more sensitive to 20- and 120-nm ZnO than male mice. One female death occurred in N2 group and one male death in N5 group, indicating that the LD_{50} of 20-nm ZnO is greater than 5-g/kg body weight and belongs to unclassified toxicity according to the Globally Harmonized Classification System (GHS) for the classification of chemicals. Such results are similar with the data of normal ZnO provided by Material Safe Data Sheet (MSDS) databases which indicates the LD_{50} of acute oral ZnO is 7950 mg/kg for mice and no death has been found during the experiment. As for 120-nm ZnO treated mice, one and three female mice died in SM2 and SM5 groups, respectively, suggesting the 120-nm ZnO was classified as GHS category 5 (2 g/kg < LD_{50} < 5 g/kg), i.e. non-toxic chemicals (Grodzki 2000). However, the LD_{50} of acute oral toxicity for 120-nm ZnO is lower than the normal ZnO.

All the dead mice in N2 and SM2 groups showed lethargy, body-weight losses and lusterless skin symptoms. Similar to the symptoms of zinc metal powder treatment (Wang et al. 2006), the symptoms such as lethargy and diarrhea appeared in the mice treated with high dose of 120-nm ZnO (5 g/kg body weight), which was in accordance with the body weight gain retardent observation (Table 3) and the stomach pathological damage (pictures will be shown later). In contrast to 120-nm ZnO treated mice, only slight lethargy and diarrhea were found in the 20-nm ZnO group mice.

In the second day after administration, the body weight (BW) of female mice in N4 and male mice in N1, N5, SM2, SM4 and SM5 groups significantly decreased ($P < 0.05$, Table 3), whereas, on day 14, the body weight regained gradually and no significant difference was found between the control and the exposed group mice except it of the female mice in SM2 group notably decreased (Table 3).

Table 2 Number of death after gastrointestinally treated with 20- and 120-nm ZnO particles ($n = 5$)

Group	N1	N2	N3	N4	N5	SM1	SM2	SM3	SM4	SM5
Dose (g/kg bw)	1	2	3	4	5	1	2	3	4	5
Number of death (F)	–	1	–	–	–	–	1	–	1	3
Number of death (M)	–	–	–	–	1	–	–	–	–	2

(F), Female mice; (M), Male mice

Table 3 Body weight gain of the mice exposed to 20- and 120-nm ZnO ($\bar{x} \pm$ S.D.)

Groups	Before		After 2 days		After 14 days	
	Male	Female	Male	Female	Male	Female
N1	21.1 ± 0.4	20.2 ± 0.7	22.5 ± 2.8*	21.5 ± 1.3	34.5 ± 0.6	26.0 ± 0.6
N2	20.9 ± 0.7	19.9 ± 1.2	24.5 ± 1.9	21.9 ± 2.8	34.1 ± 2.7	26.1 ± 1.2
N3	20.5 ± 0.7	20.2 ± 0.7	23.8 ± 2.0	21.9 ± 2.1	33.7 ± 2.5	26.2 ± 3.2
N4	20.9 ± 0.6	19.8 ± 1.0	24.8 ± 0.9	20.3 ± 1.8*	32.9 ± 2.0	26.0 ± 1.0
N5	20.7 ± 0.6	20.3 ± 0.5	21.7 ± 3.0**	21.1 ± 0.2	35.0 ± 2.9	25.5 ± 1.8
SM1	21.4 ± 0.6	20.5 ± 0.5	25.0 ± 1.2	21.5 ± 1.4	35.1 ± 3.6	24.8 ± 1.1
SM2	21.3 ± 0.6	20.3 ± 0.9	23.7 ± 2.4*	21.0 ± 2.3	34.4 ± 1.9	21.8 ± 6.9*
SM3	21.4 ± 0.7	19.8 ± 0.6	25.3 ± 1.1	21.7 ± 1.9	34.0 ± 1.1	25.1 ± 1.7
SM4	21.2 ± 0.5	19.8 ± 0.8	24.3 ± 1.1*	22.1 ± 1.4	34.4 ± 1.4	25.3 ± 1.2
SM5	21.3 ± 0.7	20.4 ± 0.6	23.4 ± 1.9**	22.1 ± 1.2	35.2 ± 3.4	27.1 ± 1.6
CT	21.2 ± 0.7	19.8 ± 0.8	26.3 ± 1.1	22.9 ± 1.3	33.4 ± 2.3	26.1 ± 1.5

* $P \leq 0.05$ vs. the control

** $P \leq 0.01$ vs. the control

Effects of 20- and 120-nm ZnO particles on serum biochemistry

The level of LDH in serum is often tested along with ALP and ALT to evaluate whether the liver is damaged or diseased. When the liver is in dysfunction, the levels of the above serum enzymes will rise (Kellerman 1995). Further investigation indicates that the significantly elevated LDH and ALT occurred in 1-g/kg body weight treated mice, indicating that liver damage might be induced by low dose of 20-nm ZnO particles (Table 4). Compared with the 20-nm ZnO treated mice, the median and high dose 120-nm ZnO exposed mice show significantly elevated levels of serum LDH and ALP, suggesting the liver damage (Table 4). It has been reported that liver damage could be induced by excess oral zinc salt and zinc powder administration (Ding et al. 1998; Chen et al. 1992, Wang et al. 2006). Ding et al. (1998) reported high dietary zinc caused liver toxicity of mice and resulted in inhibiting the activity of GOT in liver homogenate of mice.

In hospital, for diagnosis of heart disease, the elevated LDH and HBD levels are usually used to indicate the occurrence of ischemic heart diseases and acute coronary syndromes (Lee and Goldman 1986). In the study, both significantly elevated LDH and HBD levels were found in 1-g/kg body weight 20-nm ZnO treated mice (Table 4), indicating that cardiovascular diseases were possibly induced at this low dose. Similar results were observed in 120-nm ZnO treated mice as well.

Generally, compared with the data of 20-nm ZnO treated mice, the levels of the serum biochemicals are higher in 120-nm ZnO treated mice under the same dose administration (Table 4), suggesting that 120-nm ZnO may cause more severe liver damage than 20-nm ZnO.

Effects of 20- and 120-nm ZnO particles on the blood-elements and the blood coagulation

Since many epidemiological studies reported that ultrafine particulates exposure had close relationship

Table 4 Biochemical assay of serum in the mice exposed to 20- (N1–N5) and 120-nm ZnO (SM1–SM5) ($\bar{x}$ ± S.D., n = 10)

Group	LDH (U/L)	ALT (U/L)	ALP (U/L)	TP (g/L)	LAP (U/L)	TG (mmol/L)	TC (mmol/L)	UA (μmol/L)	CR (μmol/L)	P (mmol/L)	HBD (U/L)
N1	672 ± 218*	28.3 ± 2.6*	94.8 ± 10.4	43.6 ± 2.0	43.0 ± 6.1	1.4 ± 0.4	3.0 ± 0.9	125 ± 46	42.0 ± 3.2	2.7 ± 0.3	351 ± 125*
N2	466 ± 183	25.9 ± 4.9	108 ± 29	46.3 ± 3.0*	40.9 ± 3.9	1.5 ± 0.4	3.3 ± 0.7	115 ± 28	41.2 ± 1.3	2.7 ± 0.1	236 ± 97
N3	496 ± 155	20.1 ± 3.4	93.3 ± 15.3	41.8 ± 2.5	37.0 ± 3.7	1.2 ± 0.4	3.0 ± 0.7	88.1 ± 28.2	39.9 ± 3.8	2.7 ± 0.2	253 ± 89
N4	325 ± 146	22.0 ± 1.0	103 ± 46	38.4 ± 9.8	34.3 ± 9.1	1.4 ± 0.3	2.6 ± 0.8	93.8 ± 36.7	36.3 ± 9.4	2.6 ± 0.5	162 ± 71
N5	409 ± 103	19.4 ± 4.5	96.6 ± 22.8	41.8 ± 6.4	38.1 ± 9.0	1.1 ± 0.2	3.3 ± 0.4	78.3 ± 20.0	37.5 ± 3.9	2.8 ± 0.2*	199 ± 53
SM1	1018 ± 263**	21.9 ± 4.4	107 ± 26	43.0 ± 2.4	44.3 ± 4.4*	1.5 ± 0.4	2.5 ± 0.5	190 ± 77	54.0 ± 7.5**	2.5 ± 0.1	406 ± 126**
SM2	915 ± 209**	22.0 ± 3.4	133 ± 49**	40.6 ± 2.1	40.0 ± 3.2	1.3 ± 0.3	2.7 ± 0.4	181 ± 70	49.3 ± 5.5**	2.7 ± 0.3	382 ± 109*
SM3	1202 ± 281**	20.3 ± 5.2	122 ± 19**	41.6 ± 2.0	39.0 ± 6.4	1.6 ± 0.5	2.6 ± 0.4	187 ± 44**	50.4 ± 7.3**	2.8 ± 0.4	509 ± 150**
SM4	1079 ± 228**	20.9 ± 6.3	111 ± 26	41.8 ± 2.6	39.0 ± 3.5	1.6 ± 0.4	2.4 ± 0.2	168 ± 18**	45.4 ± 3.5*	2.6 ± 0.2	439 ± 106**
SM5	651 ± 194	28.9 ± 6.1*	106 ± 14	44.7 ± 3.3*	41.1 ± 4.6	1.9 ± 0.2*	3.5 ± 0.6**	91 ± 36	43.4 ± 8.6	3.2 ± 0.5*	384 ± 85**
CT	464 ± 162	23.1 ± 4.3	86.6 ± 15.6	40.7 ± 4.0	37.1 ± 1.7	1.3 ± 0.3	2.8 ± 0.4	105 ± 37	38.8 ± 4.0	2.4 ± 0.2	244 ± 85

* $P \leq 0.05$ vs. the control

** $P \leq 0.01$ vs. the control

to cardiovascular diseases (Samet et al. 2000). One of the hypothesized mechanisms is that the ultrafine particulates could cause increase of blood coagulation and then induce heart ischemia (Donaldson et al. 2003). Therefore, it is interesting to know whether there is any evidence of blood coagulation after 20- and 120-nm ZnO acute oral exposure.

In the study, the elevated RBC and HCT levels were found in N1–N4 and SM3–SM5 group mice. It is reported that an elevated RBC and HCT count may be caused by dehydration, hypoxia, or a disease called polycythemia (Nitsche 2004). In the previous study, Allen et al. (1983) found the occurrence of inappetance, diarrhoea with dehydration or subcutaneous oedema, profound weakness and jaundice in the sheep or calve after oral excess amount of Zn.

Furthermore, the decreased MCH was found in the 20- and 120-nm ZnO group mice and the decreased MCHC was observed in N2, N4 and 120-nm ZnO group, (Table 5), which is associated with anemia. Some of the previous studies also concluded that excessive dietary zinc in animals could induce deficiencies of copper and iron and then produce growth retardation and anemia (Torrance and Fulton 1987; Latimer et al. 1989; Hoffman et al. 1988; Llober et al. 1988; Hein 2003).

Platelets, also called thrombocytes, are involved in the process of blood clotting. An abnormally higher platelet count (thrombocytosis) may occur when the rate of platelet production by bone marrow increases or the removal of platelets from the blood by spleen reduces, indicating thrombus formation (Akins et al. 1996). Therefore, the significantly increased PLT counts in the 20-nm ZnO and the SM3–SM5 group mice might be a risk signal for thrombus formation (Table 5). FIB together with PT and APTT measurements are the principle regular tests for evaluation of coagulation factor changes and may be used for monitoring of the risk of cardiovascular diseases. The relatively shorter PT and APTT are associated with the increase of concentrations of particular coagulation factors what is manifested by higher risk of thrombosis (Kodama et al. 1996). The fibrinogen (FIB) level is a reflection of blood blotting ability that the elevated FIB level can be a predictor of cardiovascular diseases (Kannel et al. 1987). Table 6 presents the changes of PT, APTT and FIB after ZnO particle administration. The PT values in N5, SM1 and SM5 groups significantly decreased compared

Table 5 Blood-element test of the mice exposed to 20- (N1–N5) and 120-nm ZnO (SM1–SM5) ($\bar{x}$ ± S.D., n = 10)

Group	WBC (10^9/L)(10^9/L)	RBC (10^{12}/L)(10^{12}/L)	HCT (L/L)(L/L)	MCH (pg)(pg)	MCHC (g/l)(g/L)	PLT (10^9/L) (10^9/L)	RDW-CV	HGB (g/L)
N1	3.90 ± 0.78*	7.17 ± 0.47*	0.40 ± 0.02**	17.5 ± 0.8**	316 ± 9	866 ± 137**	0.12 ± 0.01	127 ± 7
N2	4.20 ± 0.94	7.40 ± 0.35**	0.40 ± 0.02**	17.0 ± 0.6**	313 ± 1*	834 ± 92*	0.13 ± 0.01	122 ± 5
N3	4.68 ± 1.24	7.18 ± 0.06*	0.38 ± 0.01*	17.5 ± 0.5**	321 ± 3	830 ± 127*	0.14 ± 0.01**	122 ± 4
N4	4.22 ± 1.13	7.72 ± 0.39**	0.42 ± 0.01**	16.7 ± 0.6**	309 ± 9**	881 ± 182**	0.13 ± 0.01	128 ± 3*
N5	4.40 ± 1.13	7.11 ± 0.9	0.37 ± 0.02	17.2 ± 0.4**	320 ± 5	888 ± 95**	0.14 ± 0.00**	120 ± 2
SM1	3.88 ± 0.66*	7.06 ± 0.33	0.38 ± 0.00	16.9 ± 0.1**	316 ± 9**	768 ± 216	0.12 ± 0.01	120 ± 6
SM2	4.64 ± 0.92	7.09 ± 0.26	0.39 ± 0.01**	16.8 ± 0.7*	311 ± 11**	820 ± 153	0.13 ± 0.01	122 ± 6
SM3	4.48 ± 0.75	7.13 ± 0.31*	0.38 ± 0.02**	16.9 ± 0.5**	315 ± 10**	925 ± 265*	0.13 ± 0.01	120 ± 6
SM4	7.00 ± 1.03**	7.32 ± 0.37**	0.39 ± 0.02**	16.8 ± 0.2**	314 ± 5**	879 ± 166*	0.13 ± 0.01	123 ± 8
SM5	4.78 ± 0.93	7.32 ± 0.44**	0.39 ± 0.02**	16.7 ± 0.6*	312 ± 6**	1000 ± 183**	0.22 ± 0.14	123 ± 8
CT	5.16 ± 0.90	6.69 ± 0.35	0.36 ± 0.01	18.5 ± 0.3	334 ± 13	628 ± 99	0.13 ± 0.01	122 ± 4

* $P \leq 0.05$ vs. the control

** $P \leq 0.01$ vs. the control

Table 6 Blood coagulation examination of the mice exposed to 20- (N1–N5) and 120-nm ZnO (SM1–SM5) ($\bar{x}$ ± S.D., n = 10)

Group	PT (s)	APTT (s)	FIB (mg/dL)
N1	8.87 ± 0.08	21.8 ± 1.5	209 ± 22**
N2	8.77 ± 0.24	20.2 ± 1.4*	209 ± 11**
N3	8.63 ± 0.08	24.0 ± 4.1	148 ± 23
N4	8.68 ± 0.19	20.0 ± 1.2**	195 ± 38**
N5	8.55 ± 0.16**	22.3 ± 1.1	167 ± 26
SM1	8.60 ± 0.24*	19.7 ± 0.9**	175 ± 41
SM2	8.63 ± 0.15	21.3 ± 1.1**	165 ± 11
SM3	8.85 ± 0.12	21.3 ± 1.5**	172 ± 7
SM4	8.64 ± 0.18	20.1 ± 1.0**	224 ± 86
SM5	8.30 ± 0.09**	19.0 ± 0.5**	166 ± 30
CT	8.85 ± 0.24	23.6 ± 1.7	150 ± 34

* $P \leq 0.05$ vs. the control

** $P \leq 0.01$ vs. the control

with the controls ($P < 0.05$). The time of APTT in N2, N4 and 120-nm ZnO groups was significantly reduced as well ($P < 0.05$). The FIB level in N1, N2 and N4 groups dramatically increased compared with the controls ($P < 0.05$). However, for all the above blood coagulation indicators, no obvious differences were observed between the 20- and 120-nm ZnO treated mice. The shorter PT and APTT time and the higher FIB level further suggested that thrombus and the blood viscosity increase were induced by low and median dose 20-nm ZnO. However, the significantly shortened PT and APTT times were found in high dose of 120-nm ZnO treated mice indicating that only the high dose of 120-nm ZnO has the possibility to induce thrombus in mice. The previous study has reported the significantly shortened PT and the increased blood viscosity in rabbits who supplemented with zinc gluconate (150.0 mmol/L) (Han et al. 2001).

However, for the serum biochemistry and hematology analysis, no obvious dose–response relationship was observed in the 20- and 120-nm ZnO treated mice.

Zn content analysis

The accumulation of Zn in the organic tissues and serum of the mice in N5 and SM5 groups are shown in Fig. 4. After 5 g/kg 20-nm ZnO exposure, compared with the control, significant increase of Zn contents were found in the kidney, pancreas and bone ($P < 0.05$), and slight increase was in the liver and heart of the ZnO treated mice. In contrast to the 120-nm ZnO treated mice, the Zn content in the liver, kidney and pancreas of the 20-nm ZnO group mice showed little higher, suggesting more Zn may excrete from the 20-nm ZnO administrated mice than 120-nm ZnO mice. Among the observed organs, the highest Zn content was found in the bone. The 120-nm ZnO mice retained significantly higher Zn in the bone than the 20-nm ZnO mice

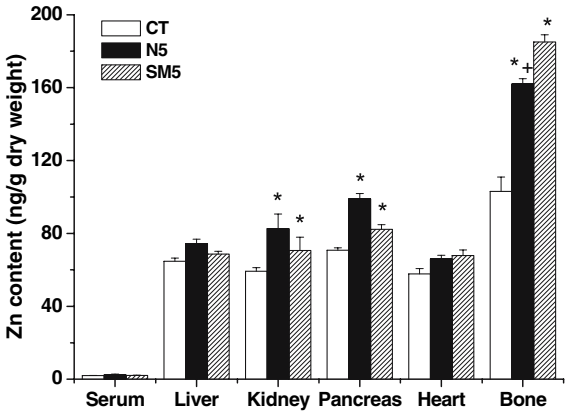

Fig. 4 Content of Zn in serum and organic tissues of mice exposed to zinc oxide powder at the dose of 5-g/kg body weight on 14-d post-oral administration. CT: Control; N5: 5-g/kg body weight 20-nm ZnO group; SM5: 5-g/kg body weight 120-nm ZnO group

($P < 0.05$). In the study of higher vertebrates and fish, bone was reported as a reservoir for dietary Zn (Gatlin et al. 1989; Sandoval et al. 1999). As for the translocation of nano-sized particle in vivo, the bone marrow was demonstrated to be one of the important target organs (Ballou et al. 2004; Cagle et al. 1999; Gibaud et al. 1996).

Pathological examination

The pathological observation shows that the lesions of stomach, liver and pancreas were found in 20-nm ZnO group mice. With the dose increase, 20-nm ZnO induced severer gastric mucosal damage, but the liver and pancreas lesion was mitigated (Table 7). In N1 group mice the heart and spleen damages were observed. For the examination of 120-nm ZnO group mice, the pathological damages of stomach, liver, heart, spleen and pancreas were found to aggravate with the dose increase, which was in accordance with the results of serum biochemical assay. Moreover, the significantly increased UA level in SM3 and SM4 group mice and the histopathological alteration of cardiovascular cells all indicated that the cardiovascular diseases occurred in 120-nm ZnO treated mice. Similar lesions were observed in the renal, pancreas and spleen of a trumpeter swan due to zinc poisoning (Carpenter et al. 2004). Our findings indicate that exposure to 20- and 120-nm ZnO may produce toxic

Table 7 Pathological alteration of the mice after 20- (N1–N5) and 120-nm ZnO (SM1–SM5) administration

Group	Stomach[a]	Liver[b]	Pancreas[c]	Kidney[d]	Spleen[e]	Heart[f]
N1	+	++	+	–	+	++
N2	+	++	+	–	–	–
N3	+	++	+	–	–	–
N4	+	+	–	–	–	–
N5	++	+	–	±	–	–
SM1	–	+	–	–	+	–
SM2	–	++	–	–	+	+
SM3	++	++	–	–	+	++
SM4	++	++	–	–	++	++
SM5	++	++	+	±	++	++
CT	–	–	–	–	–	–

[a] Inflammation in gastric lamina propria, submucosa or serosa layer

[b] Fatty degeneration of hepatocytes around central vein or portal area

[c] Inflammatory cell appears in pancreatic interstice

[d] Proteinaceous casts in renal tubule

[e] Largement of splenic corpuscle

[f] Fatty degeneration of cardiovascular cells

Note: –, no pathological alteration; ±, mild alteration; +, moderate alteration; ++, severe alteration

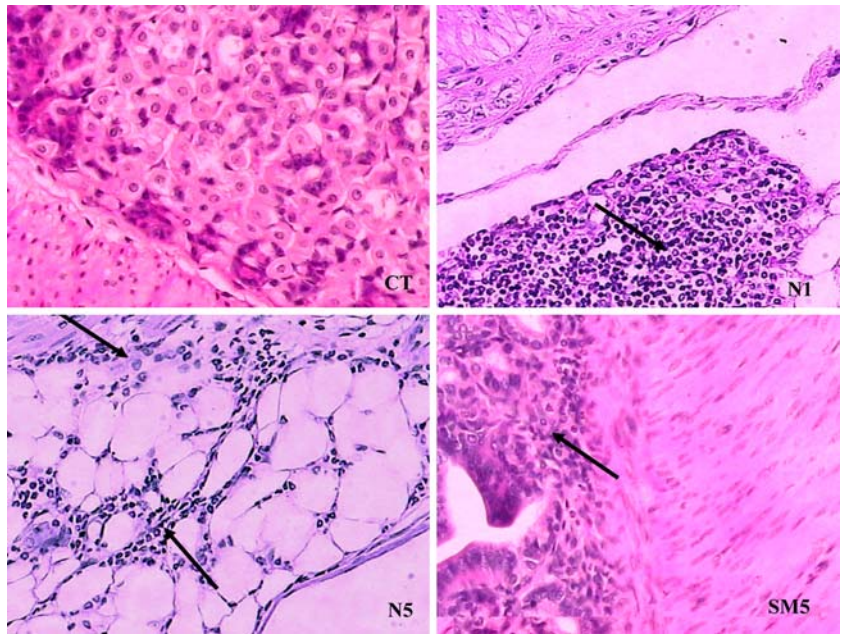

Fig. 5 Neutrophils increased in stomach of the mice exposed to 20- and 120-nm ZnO on day 14 after administration (magnification = 200). CT: Control group; N1: 1-g/kg body weight 20-nm ZnO group, the arrow shows neutrophils in the gastric serosa; N5: 5-g/kg body weight 20-nm ZnO group, arrows show neutrophils from serosa to muscularis mucosa layer; SM5: 5-g/kg body weight 120-nm ZnO group the arrow shows neutrophils in submucosa layer

effects on various tissues and organs including the gastric, liver, renal, pancreas and spleen.

Figures 5–10 show the typical histopathological alteration of stomach (Fig. 5), liver (Fig. 6), pancreas (Fig. 7), renal (Fig. 8), spleen (Fig. 9) and heart (Fig. 10) of the mice exposed to 20- and 120-nm ZnO on day 14 after administration. The gastric pathological pictures shows that the neutrophils increased in

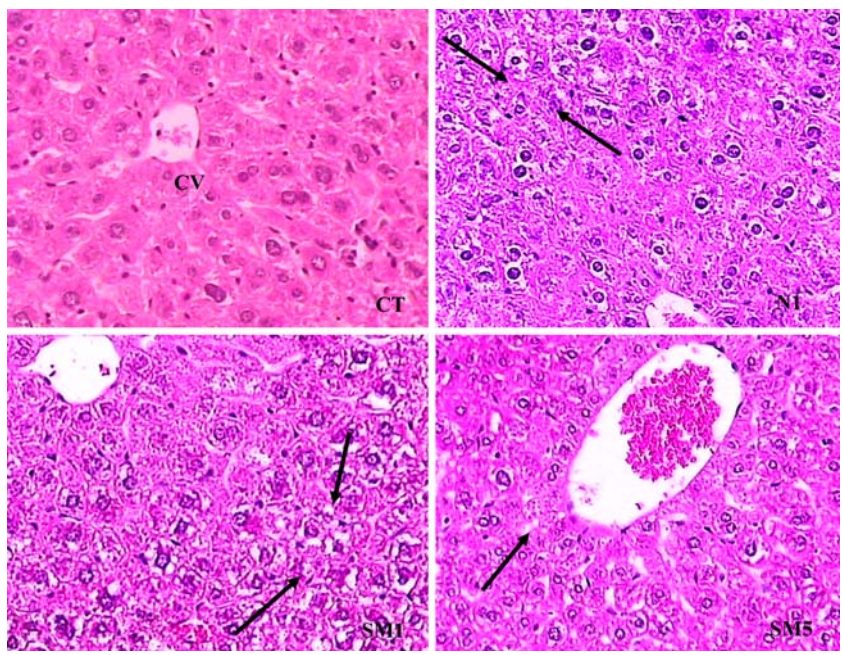

Fig. 6 Edema and degeneration in hepatocytes of the mice exposed to zinc oxide powder on 14-d post-oral administration (magnification = 200). CT: Control group; N1: 1-g/kg body weight 20-nm ZnO group, the arrow shows edema and degeneration of hepatocytes on the marginal region of liver lobule; SM1: 1-g/kg body weight 120-nm ZnO group, arrows show edema and degeneration of hepatocytes in the portal area of liver; SM5: 5-g/kg body weight 120-nm ZnO group, the arrow shows edema and degeneration of hepatocytes presented around central vein

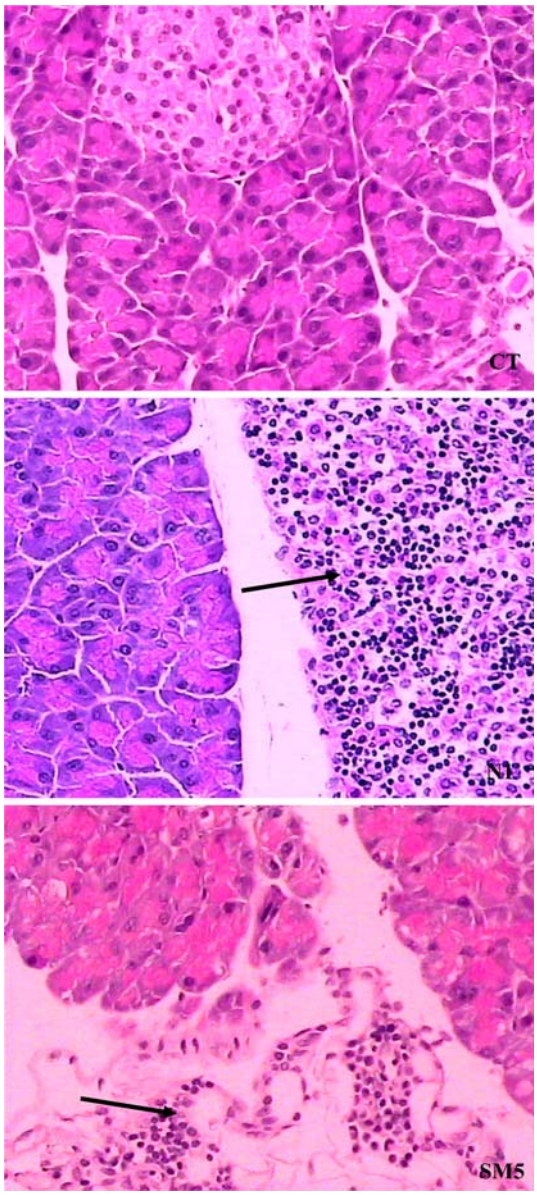

Fig. 7 Pancreas pathological changes of the mice exposed to zinc oxide powder on 14-d post oral administration (magnification = 200). CT: Control group; N1: 1-g/kg body weight 20-nm ZnO group, the arrow shows chronic inflammatory cells and lymphocytes of pancreas interstice; SM5: 5-g/kg body weight 120-nm ZnO group, the arrow shows inflammatory cells infiltration in pancreas interstice

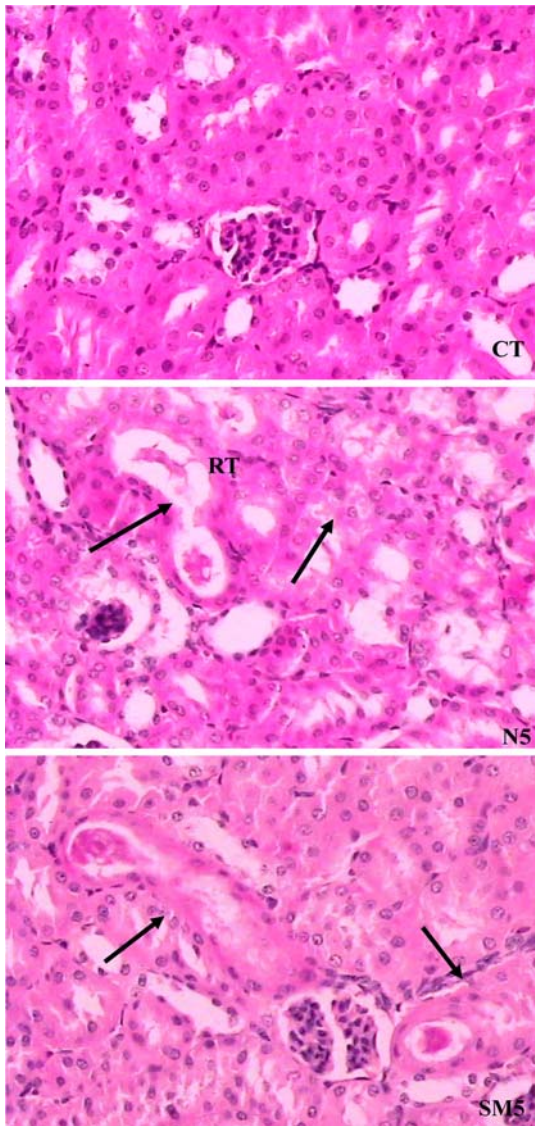

Fig. 8 Renal pathological changes of the mice exposed to zinc oxide powder on 14-d post-oral administration (magnification = 200). CT: Control group; N5: 5-g/kg body weight 20-nm ZnO group, the arrows show a smack of proteinaceous casts in renal tubule; SM5: 5-g/kg body weight 120-nm ZnO group, the arrows show a spot of proteinaceous casts in renal tubule. RT: renal tubular

the serosa layer of the stomach in 20-nm ZnO mice and the lamina propria and submucosa layer of the 120-nm ZnO mice (Fig. 5). Such gastric damage exhibited a dose–effect relationship for both 20- and 120-nm ZnO particle treatment. Furthermore, significant hydropic degeneration in the hepatocytes were found in 20- and 120-nm ZnO treated mice and the chronic inflammatory cells were found in the

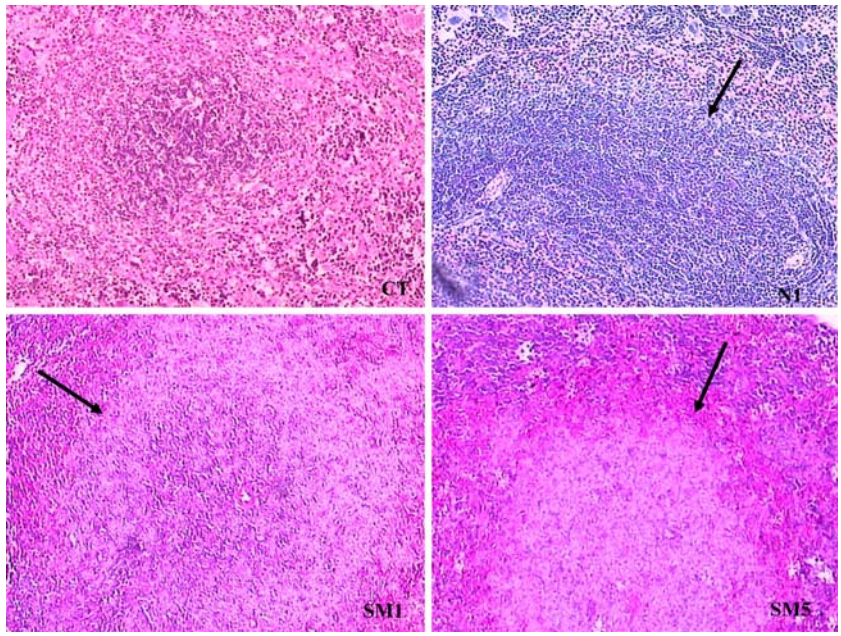

Fig. 9 Enlargement of splenic corpuscle of the mice exposed to zinc oxide on day 14 after administration (magnification = 100). CT: Control group; N1: 1-g/kg body weight 20-nm ZnO group; SM1: 1-g/kg 120-nm ZnO; SM5: 5-g/kg body weight 120-nm ZnO group. The arrow shows severe enlargement of splenic corpuscle

pancreas of N1–N3 and SM5 group mice. Figure 8 shows proteinaceous casts in renal tubule of the mice administrated by 5-g/kg body weight 20- and 120-nm ZnO particles. Slight enlargement of the splenic corpuscle was found in N1 and 120-nm ZnO group mice (Fig. 9). Figure 10 shows fatty degeneration in cardiovascular cells was induced by 1-g/kg body weight 20- and 120-nm ZnO treatment.

Besides the above organic tissues, no other significant histopathological changes were found in the lung, testis, uterus and the brain.

The negative effect of 20-nm ZnO in the mice may be explained by the 20-nm ZnO particles at high dose easier to agglomerate in vivo. Since it has been suggested that the biological effects of nano-scaled particles might mainly contribute to their physicochemical properties, such as size effect, surface area and surface charge, etc., (Oberdörster et al. 1994; Oberdörster et al. 2005; Long et al. 2006), which means that particle number or surface area might be undertaken more important role than the mass concentration in the toxicological study (Teeguarden et al. 2007; Stoeger et al. 2006; Wittmaack 2006; Warheit et al. 2006). Therefore, results from our and previous studies, further study about the biological or toxic effect of nanomaterials might be better expressed by the particle number or surface area.

Conclusions

The acute oral toxicity study of 20- and 120-nm ZnO particles indicates that 20- and 120-nm ZnO, similar to the normal ZnO compound, are relatively nontoxic for mice according to GHS classification criteria. Combined with the results of zinc accumulation, pathological examination and the biological indicators assays, the target organs for 20- and 120-nm ZnO acute oral administration are demonstrated as liver, heart, spleen, pancreas and bone. The biochemical and pathological investigation shows that the toxic effects between the 20- and 120-nm ZnO particles are a little different. For example, the blood viscosity could be induced by low and median dose of 20-nm ZnO but high dose of fine ZnO after oral administration. The edema and degeneration of hepatocytes, and inflammation of pancreas could be observed in most of the 20-nm ZnO treated mice. The 120-nm ZnO treated mice were found having dose–effect pathological damage in gastric, liver, heart and spleen, however, the 20-nm ZnO treated mice

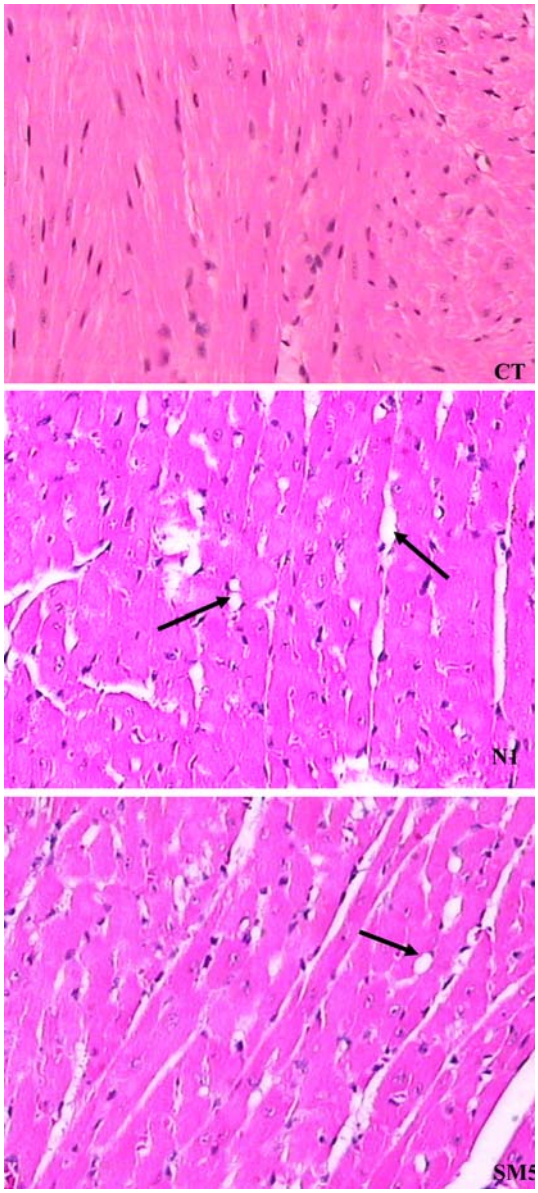

Fig. 10 Fatty degeneration in cardiovascular cells of the mice exposed to zinc oxide powder on 14-d post-oral administration (magnification = 200). CT: Control group; N1: 1-g/kg body weight 20-nm ZnO group; SM5: 5-g/kg body weight 120-nm ZnO group. The arrows show fatty degeneration in cardiovascular cells

presented lessened liver, spleen and pancreas damage with the increase of treated dose. Therefore, more attention should be paid on the potential toxicity induced by low dose of small-sized 20-nm ZnO oral exposure.

Acknowledgements The authors are grateful to the foundations of National Basic Research Program of China (2006CB705605), National Natural Science Foundation of China (10490180, 10675139) and the Chinese Academy of Sciences (Grant No. KJCX2-SW-N01) and the special foundation for excellent doctoral dissertation.

References

Akins PT, Glenn S, Nemeth PM, Derdeyn CP (1996) Carotid artery thrombus associated with severe iron-deficiency anemia and thrombocytosis. Stroke 27:1002–1005

Ballou B, Lagerholm BC, Ernst LA, Bruchez MP, Waggoner AS (2004) Non-invasive imaging of quantum dots in mice. Bioconjugate Chem 15:79–86

Banerjee S, Dan A, Chakravorty D (2002) Review synthesis of conducting nanowires. J Mater Sci 37:4261–4271

Cagle DW, Kenmnel SJ, Mirzadeh S, Alford JM, Wilson LJ (1999) In vivo studies of fullerene based materials using endohedral metallofullerene radiotracers. Proc Natl Acad Sci 96:5182–5187

Carpenter JW, Andrews GA, Beyer WN (2004) Zinc toxicosis in a free-flying trumpeter swan (cygnus buccinator). J Wildlife Dis 40(4):769–774

Chen RH, Qin R, Wang FD, Wang JP, Lu TX (1992) The effects of oral excess zinc on the zinc level and morphology of tissues. Zhonghua Yixue Zazhi 72(7):391–393

Ding H, Peng R, Chen J (1998) Effects of high dietary zinc on liver function, hepatic drug metabolism enzymes and membrane fluidity in mice. Wei Sheng Yan Jiu 27(3):180–182

Donaldson K, Stone V (2003) Current hypotheses on the mechanisms of toxicity of ultrafine particles. Ann Ist Super Sanit'a 39(3):405–410

Gatlin III DM, Phillips HF, Torrans EL (1989) Effects of various levels of dietary copper and zinc on channel catfish. Aquaculture 76:127–134

Gibaud S, Demoy M., Andreux JP, Weingarten C, Gouritin B, Couvreur P (1996) Cells involved in the capture of nanoparticles in hematopoietic organs. J Pharm Sci 85(9):944–950

Grodzki K (2000) Establishing a globally harmonised hazard classification and labelling system for dangerous substances and preparations. Tutb Newsletter 14:17–23

Han CY, Bian JC, Yang XX, Wang L, Zhang HF, Xiang YZ, Shi BE (2001) The effects of Zn on blood viscosity and Prothormbin time. Studies of Trace Elements and Health 18(1):13–14

Hein MS (2003) Copper deficiency anemia and nephrosis in zinc-toxicity: a case report. S D J Med 56(4):143–147

Hoffman HN, Phyliky RL, Fleming CR (1988) Zinc-induced cop-per deficiency. Gastroenterology 94(2):508–512

Kannel WB, Wolf PA, Castelli WP, D'Agostino RB (1987) Fibrinogen and risk of cardiovascular disease. The Framingham Study. JAMA 258(9):1183–1186

Kellerman J (1995) In: Blood test. Signet Book, Reprint edition, Chicago, USA

Kodama H, Fukuda J, Karube H, Matsui T, Shimizu Y, Tanaka T (1996) Status of the coagulation and fibrinolytic system

in ovarian hyperstimulation syndrome. Fertil Steril 66(3):417–424

Lam CW, James JT, McCluskey R, Hunter RL (2004) Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. Toxicol Sci 77:126–134

Latimer KS, Jain AV, Inglesby HB, Clarkson WD, Johnson GB (1989) Zinc-induced hemolytic anemia caused by ingestion of pennies by a pup. J Am Vet Med Assoc 195(1):77–80

Lee CJ, Lee TJ, Lyu SC, Zhang Y, Ruh H, Lee HJ (2002) Field emission from well-aligned zinc oxide nanowires grown at a low temperature. Appl Phys Lett 81(19):3648–3650

Lee DD, Lee DS (2001) Environmental gas sensors. IEEE Sens J 1(3):214–224

Lee TH, Goldman L (1986) Serum enzyme assays in the diagnosis of acute myocardial infarction. Ann Intern Med 105:221–223

Llobet JM, Domingo JL, Colomina MT, Mayayo E, Corbella J (1988) Subchronic oral toxicity of zinc in rats. Bull Environ Contam Toxicol 41:36–43

Long T, Saleh N, Tilton RD, Lowry GV, Veronest B (2006) Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity. Environ Sci Technol 40(14):4346–4352

Nitsche EK (2004) Erythrocytosis in dogs and cats: diagnosis and management. Compend. Cont Educ Pract Vet 26:104–118

Oberdörster G, Ferin J, Lehnert BE (1994) Correlation between particle size, in vivo particle persistence and lung injury. Environ Health Perspect 102:173–179

Oberdörster G, Finkelstein JN, Johnston C (2000) Acute pulmonary effects of ultrafine particles in rats and mice. Res Rep Health Eff Inst 96:5–74

Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S, Monteiro-Riviere N, Warheit D, Yang H (2005) Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol 6:2(8)

OECD (2001) OECD Guidelines for Testing of Chemicals. No 425: Acute Oral Toxicity-UP-and-Down Procedure. Organisation for Economic Co-operation and Development, Paris, Adopted: 17th December

Pekkanen J, Peters A, Hoek G, Tiittanen P, Brunekreef B, de Hartog J, Heinrich J, Ibald-Mulli A, Kreyling WG, Lanki T, Timonen KL, Vanninen E (2002) Particulate air pollution and risk of ST-segment depression during repeated submaximal exercise tests among subjects with coronary heart disease: the exposure and risk assessment for fine and ultrafine particles in ambient air [ultra] study. Circulation 106:933–938

Penttinen P, Timonen KL, Tiittanen P, Mirme A, Ruuskanen J, Pekkanen J (2001) Ultrafine particles in urban air and respiratory health among adult asthmatics. Eur Resp J 17(3):428–435

Peters A, Doring A, Wichmann HE, Koenig W (1997a) Increased plasma viscosity during an air pollution episode: a link to mortality? Lancet 349:1582–1587

Peters A, Wichmann HE, Tuch T, Heinrich J, Heyder J (1997b) Respiratory effects are associated with the number of ultrafine particles. Am Respir Crit Care Med 155:1376–1383

Qiang JL (2001) The surface properties and photocatalytic activities of ZnO ultrafine particles. Appl Surf Sci 180:308–314

Rahman Q, Lohani M, Dopp E, Pemsel H, Jonas L, Weiss DG, Schiffman D (2002) Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in syrian hamster embryo fibroblasts. Environ Health Perspect 110:797–800

Samet JM, Dominici F, Curriero FC, Coursac I, Zeger SL (2000) Fine particulate air pollution and mortality in 20 U.S. cities, 1987–1994. N Engl J Med 343(24):1742–1749

Sandoval M, Henry PR, Littell RC, Miles RD, Butcher GD, Ammerman DB (1999) Effect of dietary zinc source and method of oral administration on performance and tissue trace mineral concentration of broiler chicks. J Anim Sci 77:1788–1799

SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS (SCCNFP.) (2003) Evaluation and opinion on: Zinc oxide. 24th plenary meeting, Brussels

Stoeger T, Reinhard C, Takenaka S, Schroeppel A, Karg E, Ritter B, Heyder J, Schulz H (2006) Instillation of six different ultrafine carbon particles indicates a surface area threshold dose for acute lung inflammation in mice. Environ Health Perspect 114:328–333

Teeguarden JG, Hinderliter PM, Orr G, Thrall BD, Pounds JG (2007) Particokinetics in vitro: dosimetry considerations for in vitro nanoparticle toxicity assessments. Toxicol Sci 95(2):300–312

The Royal Society & the Royal Academy of Engineering (2004) Nanoscience and nanotechnologies: opportunities and uncertainties. 29 July

Torrance AG, Fulton RB Jr (1987) Zinc-induced hemolytic anemia in a dog. J Am Vet Med Assoc 191(4):443–444

Von Klot S, Wolke G, Tuch T, Heinrich J, Dockery DW, Schwartz J, Kreyling WG, Wichmann HE, Peters A (2002) Increased asthma medication use in association with ambient fine and ultrafine particles. Eur Respir J 20:691–702

Wang B, Feng WY, Wang TC, Jia G, Wang M, Shi JW, Zhang F, Zhao YL, Chai ZF (2006) Acute toxicity of nano- and micro-scale zinc powder in healthy adult mice. Toxicol Lett 161(2):115–123

Warheit DB, Webb TR, Sayes CM, Colvin VL, Reed KL (2006) Pulmonary instillation studies with nanoscale TiO_2 rods and dots in rats: toxicity is not dependent upon particle size and surface area. Toxicol Sci 91:227–236

Wichmann HE, Cyrys J, Stölzel M, Spix C, Wittmaack K, Tuch T, Pitz M, Peters A, Wölke G, Menzel N, Hietel B, Schulz F, Heinrich J, Kreyling WG, Heyder J (2002) Sources and elemental composition of ambient particles in Erfurt, Germany. In: Wichmann HE, Schlipkoter HW, Fulgraff G (eds) Fortschritte in der Umweltmedizin. Ecomed Publishers, Erfurt, Germany

Wittmaack K (2006) In Search of the most relevant parameter for quantifying lung inflammatory response to nanoparticle exposure: particle number, surface area, or what? Environ Health Perspect 114:187–194