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Toxicology Letters 161 (2006) 115–123
Toxicology Letters
www.elsevier.com/locate/toxlet

Acute toxicity of nano- and micro-scale zinc powder in healthy adult mice

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Received 30 April 2005; received in revised form 5 August 2005; accepted 5 August 2005
Available online 13 September 2005

Abstract

The purpose of this study is to evaluate the acute toxicity of oral exposure to nanoscale zinc powder in mice. The healthy adult male and female mice were gastrointestinally administered at a dose of 5 g/kg body weight with two size particles, nanoscale zinc (N-Zn) and microscale zinc (M-Zn) powder, while one group mice treated with sodium carboxy methyl cellulose was used as the control. The symptoms and mortality after zinc powder treatment were recorded. The effects of particles on the blood-element, the serum biochemical level and the blood coagulation were studied after 2 weeks of administration. The organs were collected for histopathological examination. The N-Zn treated mice showed more severe symptoms of lethargy, vomiting and diarrhea in the beginning days than the M-Zn mice. Deaths of two mice occurred in the N-Zn group after the first week of treatment. The mortalities were confirmed by intestinal obstruction of the nanoscale zinc aggregation. The biochemical liver function tests of serum showed significantly elevated ALT, AST, ALP, and LDH in the M-Zn mice and ALT, ALP, and LDH in the N-Zn mice compared with the controls ($P < 0.05$), which indicated that the liver damage was probably induced by both micro- and nano-scale zinc powders. The clinical changes were observed in the two treated group mice as well. The levels of the above enzymes were generally higher in the M-Zn mice than in the N-Zn mice, which implied that M-Zn powder could induce more severe liver damage than N-Zn. The biochemical renal function tests of serum BUN and CR in the M-Zn mice markedly increased either compared with the N-Zn mice or with the controls ($P < 0.05$), but no significant difference was found between the N-Zn and the control mice. However, severe renal lesions were found by the renal histopathological examination in the N-Zn exposed mice. Therefore, we concluded that severe renal damage could occur in the N-Zn treated mice, though no significant change of blood biochemical levels occurred. Blood-element test showed that in the N-Zn mice, PLT and RDW-CV significantly increased, and HGB and HCT significantly decreased compared to the controls, which indicated that N-Zn powder could cause severe anemia. Besides the pathological lesions in the liver, renal, and heart tissue, only slight stomach and intestinal inflammation was found in all the zinc treated mice, without significant pathological changes in other organs.

Keywords: Acute toxicity; Nanoscale zinc powder; Microscale zinc powder; Mice

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doi:10.1016/j.toxlet.2005.08.007

1. Introduction

Zinc (Zn) is widely used in brass, bronze, die casting metal, alloys, rubber, and paints, etc. (Barceloux, 1999). In recent years, nanoscale metal powders, because of their large specific surface area, ultrahigh reactive surface sites and quantum effects, are being widely used in the traditional industries, such as dyes, paints, medical diagnosis, sunscreens and cosmetics, etc. (Colvin, 2003). Up to now, nano Zn powder is usually used as highly reactive catalyst in organic hydrogen reaction, automobile tail gas disposal, environmental protection, paints, pigment, metallurgy additives, etc. It is reported that the catalytic capabilities of nanoscale metal powder are dozens higher than the larger one (Colvin, 2003).

With the industrialization of the nanotechnology, public exposure to nanoparticles should be increasing in the near future. But so far, it is unknown what their health impacts are when nanoparticles enter into human body directly during manufacturing processes or indirectly via the environment (such as aerosol with nanoparticles) and food chains, etc. It is reported that U.S. Department of Agriculture (USDA) is researching the use of nanoparticles in food packaging and ultimately in food itself (ETC Group, 2003).

Acute, high-dose oral exposure to zinc and its compounds generally results in gastrointestinal damage, with symptoms including nausea, vomiting, abdominal cramps, and diarrhea. Exposure levels resulting in these effects generally range from 2 to 8 mg zinc/kg/day. Ingesting high levels of zinc for several months may cause anemia, pancreas damage, and decrease the level of high-density lipoprotein (HDL) cholesterol (U.S. Department of Health and Human Services, 2003).

Zn is, however, an essential metal needed by mammals and commonly found in nutritional supplements. It is necessary for the functions of a large number of metalloenzymes, DNA synthesis, cell growth and division, membrane metabolism, brain development and so forth. The requirement amount of Zn is recommended as 10–20 mg/day/person. Zn deficiency causes a variety of disorders, including growth retardation, cancer, infections, skin diseases, and slowly wound healing (U.S. Department of Health and Human Services, 2003; Barceloux, 1999).

Generally, the concentrations or particle number of natural nanoscale zinc and its compounds are usually very low in the environment, but with the elevation of manufacture and widely commercial use, the possibility of exposure to high concentrations of nanoscale Zn materials is likely to increase. So a question arises whether there is any special toxicity of nanoscale materials compared with the normal-sized ones (>100 nm). Currently, some investigations have found that exposure to ultrafine (<100 nm) particles could cause tissue inflammation, pulmonary damage, such as lung tumours and fibrosis (Donaldson et al., 2001, 2004; Zhou et al., 2003; Rahman et al., 2002; Oberdörster et al., 1992, 1994). For example, TiO_2 is considered as biologically inert; however, Oberdörster et al. (1994) observed that 20 nm TiO_2 generated more severe injury to macrophages than 250 nm particles did. The U.S. Army reported that the safe inhalation exposure limits for TiO_2 nanoparticles were at least eight times lower than normal TiO_2 particles (CLS, 1999).

The potential hazard of high concentration of manufactured nanoscale zinc powder is still unknown and their toxicological data are rather sparse, although the toxicity of normal zinc and zinc compounds has been much reported (Lock and Janssen, 2003; Piao et al., 2003; Houston et al., 2001; Talcott, 2001; Llobet et al., 1988; Chandra, 1984). The aim of this study is to evaluate the acute oral toxicity of nanoscale Zn powder according to the guidelines of OECD (OECD, 1992). In the meantime, as a contrast, the microscale zinc powder was studied as well. Additionally, the effects of particles on the blood-element, the serum biochemical level, the blood coagulation and the histopathological examination were also investigated.

2. Materials and methods

2.1. Nano- and micro-scale zinc particles

2.1.1. Particle size

Two size zinc metal powders were used in the acute toxic experiments. The microscale zinc powder was purchased from Haoyun Industrial and Trade Co. Ltd, Beijing, China, and the nanoscale powder was from Zunye Nanomaterials Co. Ltd, Shenzhen, China. The size of the microscale and nanoscale Zn particle was determined to be 1.08 ± 0.25 μm and 58 ± 16 nm diameter, respectively, by transmission electron microscopy (TEM, JEM 200CX, Fig. 1).

2.1.2. Particle purity

The metal particles were dissolved in concentrated nitric acid (MOS grade, Institute of Chemical Reagent, Beijing, China) and the element impurities were determined by ICP-AES (Baird ICP2070, USA). The analytical results show that the purity of the two size zinc powder is more than 99.99 %.

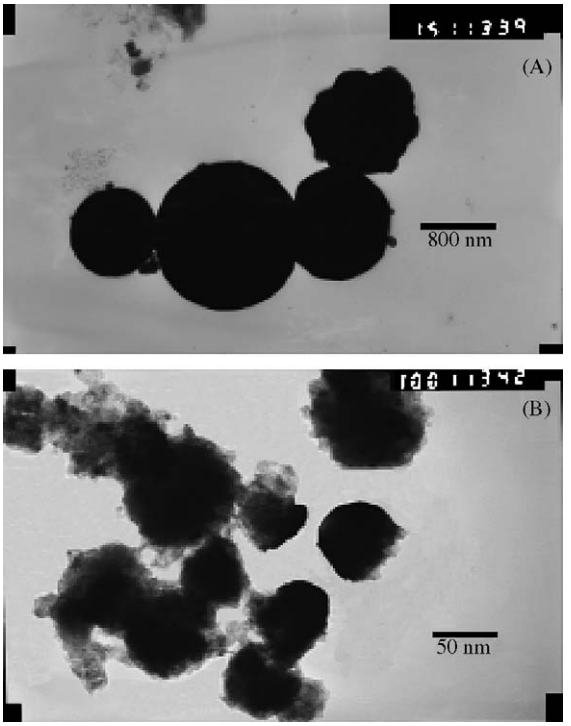

Fig. 1. (A) TEM image for microscale zinc powder; (B) TEM image for nanoscale zinc powder.

2.1.3. Preparation of particle suspension

The administrated particles were suspended in 1% sodium carboxy methyl cellulose and dispersed by ultrasonic vibration for 15 min. The concentration of Zn in the suspension was 200 mg/mL. In order to avoid the aggregation of the particles, a few glass beads were added in the suspension and then stirred on vortex agitator before every use.

2.2. Animals and treatment

Animal test was performed with compliance of the local ethics committee. Sixty CD-ICR mice of about 4 weeks old and 18–20 g weight, male and female each half, were supplied by the Experimental Animal Center, Peking University. The animals were housed by sex and maintained on commercial pellet diet, given deionized water ad libitum and kept in plastic cages in a $20 \pm 2\,^{\circ}C$, 50–70% relative humidity room with a 12-h light/dark cycle. After 1 week acclimation, the mice were randomly divided into three groups: microscale zinc (M-Zn), nanoscale zinc (N-Zn) and the control group. Each group had 10 male and 10 female mice. The animals were kept fasting over night before treatment. The M-Zn group mice was gastrointestinally administered by M-Zn suspension at a dose of 5 g Zn/kg body weight, and the N-Zn group was given by nanoscale zinc at a dose of 5 g Zn/kg body weight. The control group was given by 1% sodium carboxy methyl cellulose solution, instead. After administration, mice were observed daily for total 14 days. The symptoms of observation included changes in skin and fur, eyes membranes, respiratory, circulatory, autonomic and central nervous systems, and behaviour pattern. Special attention was given to tremors, convulsions, salivation, nausea, vomiting, diarrhoea, lethargy, sleep and coma. The body weight of mice was recorded before and every 3 days after the administration.

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

2.3. Blood assay

2.3.1. Biochemical assay of serum

The serum was obtained by centrifugation of the whole blood at 3000 rpm for 15 min. The biochemical levels including lactate dehydrogenase (LDH), alanine aminotransferas (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), cholesterol esters (CHE), total proteins (TP), albumin (ALB), blood urea nitrogen (BUN), creatinine (CR), creatine kinase (CK), hydroxybutyrate dehydrogenase (HBD) were assayed by an automatic biochemical analyzer (7170A, Hitachi, Tokyo).

2.3.2. Blood-element test and blood coagulation examination

0.1 mL of 15 g/L EDTA-Na was added into 1 mL whole blood and the sample was immediately analyzed. The blood-element: white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean cell hemoglobin concentration (MCHC), red cell distribution width corpuscular volume (RDW-CV) and blood platelet (PLT) were determined by an automatic hematology analyzer (SYSMEX Co. Ltd kx-21N, Japan).

0.1 mL of 3.8% sodium citrate was added into 1 mL whole blood sample and the blood plasma was separated by centrifugation at 5000 rpm for 5 min. The levels of prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen (FIB), which are related to blood coagulation in plasma, were determined by enzyme-linked immunosorbent assay (ELISA).

2.4. Histopathological observation

A small piece of heart, liver, kidney, spleen, lung, pancreas, stomach, small intestine, and brain was fixed by 10% formalin and then embedded into paraffin, sectioned for 5 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.

2.5. Statistical analysis

For statistical analysis, each of the experimental values was compared to its corresponding control ones, and the N-Zn values were compared to its corresponding M-Zn ones as well. A one-way analysis of variance (ANOVA) and the L.S.D. or Tamhane test was used to compare the means of the control group and each of the groups exposed to Zn powders. A significant difference was considered to be $P < 0.05$.

3. Results

3.1. Symptoms and mortality

The mice gastrointestinally administrated with 5 g/kg body weight nanoscale zinc suspensions showed severe symptoms of lethargy, anorexia, vomiting, and diarrhea in the beginning days after treatment. However, only slight lethargy was found in M-Zn treated mice. One week later, all the symptoms of the two group mice gradually disappeared. At 2 and 6 days after administration, one female and one male mice treated with N-Zn were dead, respectively. Two mortalities were preceded by anorexia, lethargy, body-weight losses, and lustreless skin. Further post-mortem investigation demonstrated that the mortalities were caused by severe aggregation of zinc particles obstruction in the intestine (see Fig. 2). But no death occurred in the M-Zn group.

Fig. 2. Instestinal obstruction observed in mice treated with 5 g/kg body weight nanoscale zinc powder.

Table 1
Body weights of mice ($\bar{x} \pm$ S.D., $n = 16$)

	N-Zn	M-Zn	Control
Before administration			
Male (g)	18.1 ± 0.1	19 ± 0.9	18.8 ± 0.3
Female (g)	18.1 ± 0.1	18.0 ± 0.1	18.1 ± 0.2
3 days after administration			
Male	19.3 ± 3.3[a,b]	23.1 ± 1.5	25.0 ± 1.4
Female	17.6 ± 1.7[a,b]	21.4 ± 2.1	22.3 ± 1.4
14 days after administration			
Male (g)	29.8 ± 4.1	32.3 ± 2.0	31.6 ± 2.5
Female (g)	22.7 ± 1.0	24.2 ± 1.8	24.8 ± 2.3

Before administration: male, $n = 10$; female, $n = 10$; 3 days after administration: N-Zn: male, $n = 10$; female, $n = 9$. M-Zn: male, $n = 10$; female, $n = 10$; 14 days after administration: N-Zn: male, $n = 9$; female, $n = 9$. M-Zn: male, $n = 10$; female, $n = 10$.

[a] Significant difference between nanoscale zinc treated mice and the control, $P < 0.05$.

[b] Significant difference between nanoscale zinc treated mice and the microscale zinc, $P < 0.05$.

The body weight gain of the mice in the N-Zn group was about 22% lower than the control mice at the first three days after the treatment, however, in the same time, no significant difference of the body weight was found between the M-Zn and the control mice (Table 1). The N-Zn mice re-gained their body weights at 6 days after treatment.

3.2. Effects of nano- and micro-scale zinc particles on serum biochemistry of mice

The effects of oral administration of zinc particles on the serum biochemical levels of mice are shown in Table 2. The results indicated that the serum LDH, ALT,

Table 2
Biochemical assay in serum ($\bar{x} \pm$ S.D., $n = 16$)

Parameters	N-Zn	M-Zn	Control
LDH (U/L)	781 ± 208[a]	962 ± 239[a]	494 ± 88
ALT (U/L)	23.7 ± 3.8[a]	25.4 ± 7.8[a]	17.8 ± 2.7
AST (U/L)	67 ± 19	77 ± 19[a]	57 ± 16
ALP (U/L)	119 ± 36[a]	119 ± 29[a]	91 ± 18
CHE (U/L)	31.9 ± 6.7[b]	37.9 ± 5.0[a]	32.1 ± 5.8
TP (g/L)	37.5 ± 4.2[b]	42.2 ± 2.2[a]	35.2 ± 4.9
ALB (g/L)	24.3 ± 2.4[b]	26.7 ± 2[a]	23.2 ± 2.6
BUN (mmol/L)	6.4 ± 1.8	6.9 ± 1.1[a]	5.5 ± 0.7
CR (μmol/L)	42.8 ± 9.2	43.2 ± 7.3[a]	37.8 ± 3.4
CK (U/L)	345 ± 191	401 ± 149	337 ± 83
HBD (U/L)	394 ± 98[a,b]	541 ± 191[a]	256 ± 56

[a] Significant difference between microscale or nanoscale zinc treated mice and the control, $P < 0.05$.

[b] Significant difference between nanoscale and microscale zinc treated mice, $P < 0.05$.

ALP, and HBD of both the M-Zn and the N-Zn mice were significantly higher than the controls ($P < 0.05$). Compared with the N-Zn mice, the levels of the above enzymes were generally higher in the M-Zn mice. The levels of the serum AST, CHE, TP, ALB, BUN, and CR in the M-Zn mice markedly increased either compared with the N-Zn or with the control mice ($P < 0.05$), however, no significant difference was found between the N-Zn and the control mice. The values of CHE, TP, ALB, and HBD in the N-Zn mice were found significantly lower than in the M-Zn mice.

3.3. Effects of nano- and micro-scale zinc particles on the blood-elements and the blood coagulation

The results presented in Table 3 showed that the levels of RDW-CV and PLT in the N-Zn mice markedly increased, while HGB and HCT in the N-Zn mice obviously decreased versus the controls ($P < 0.05$). In the M-Zn group, the levels of HCT also decreased compared with the controls. When compared with N-Zn mice, more decrease of HCT and HGB were observed in the N-Zn mice than in the M-Zn ones. However, PT, FIB, and APTT showed no significant difference among the three group mice.

3.4. Histopathological examination

The histopathological examination found the slight stomach and intestinal inflammation in almost all the nano and micro Zn administrated mice.

The liver histopathological pictures are illustrated in Fig. 3. At the microscopic level, the liver of mice in both

Table 3
Blood-element test and blood coagulation examination ($\bar{x} \pm$ S.D., $n = 6$)

Parameters	N-Zn (nm)	M-Zn (μm)	Control
WBC (10^9/L)	5.3 ± 1.4	5.9 ± 0.9	5.7 ± 0.6
RBC (10^{12}/L)	7.0 ± 0.2	6.8 ± 0.5	7.2 ± 0.3
HGB (g/L)	127 ± 2[a]	130 ± 8	138 ± 5
HCT (L/L)	0.47 ± 0.01[a,b]	0.44 ± 0.02[a]	0.49 ± 0.01
MCV (10^{-15} L)	67.3 ± 2.2	65.7 ± 2.1	67.3 ± 1.0
MCHC (g/L)	278 ± 9	289 ± 9	281 ± 13
RDW-CV	0.135 ± 0.009[a]	0.125 ± 0.004	0.125 ± 0.004
PLT (10^9/L)	988 ± 200[a,b]	756 ± 121	781 ± 113
PT (s)	8.89 ± 0.29	8.96 ± 0.29	9.24 ± 0.48
FIB (mg/dL)	165 ± 15	156 ± 32	167 ± 36
APTT (s)	21.7 ± 1.7	21.4 ± 1.6	22.1 ± 1.5

[a] Significant difference between microscale or nanoscale zinc treated mice and the control, $P < 0.05$.

[b] Significant difference between nanoscale and microscale zinc treated mice, $P < 0.05$.

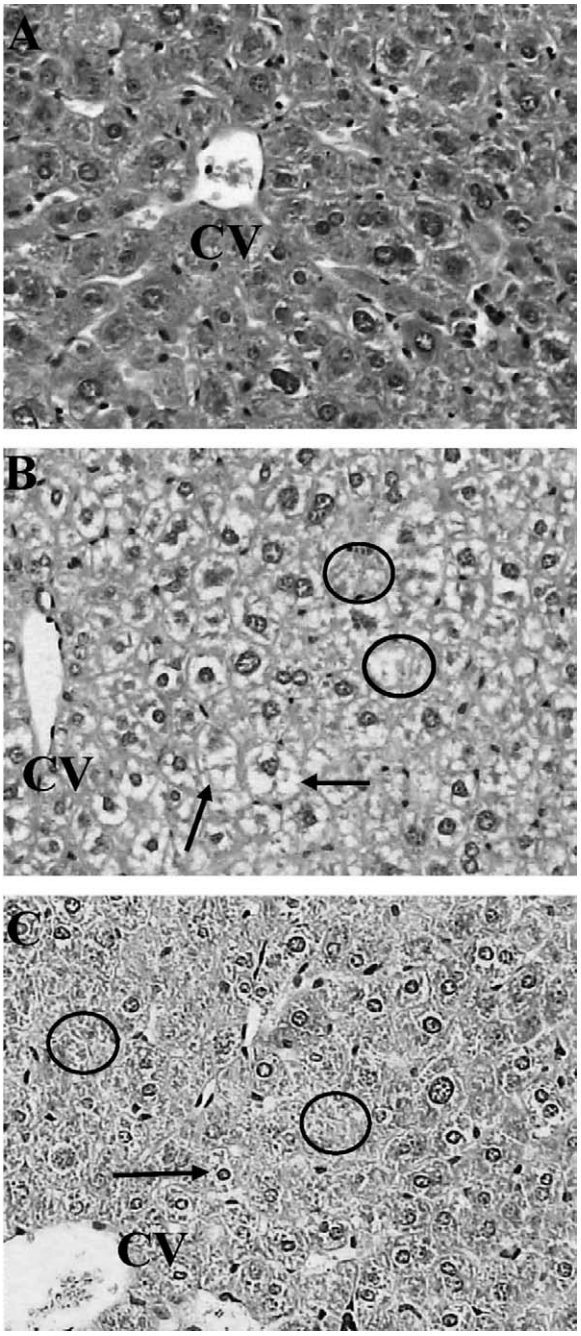

Fig. 3. Liver tissue from mice exposed to zinc powder at an acute toxic dose of 5 g/kg body weight on 14 days post-oral administration (magnification = 200). (A) Control group (instilled 1% sodium carboxy methyl cellulose). CV: central vein. (B) Microscale group. Arrows show the hydropic degeneration of hepatocytes. Circle areas show the slight necrosis of hepatocytes. (C) Nanoscale group. Arrows show the hydropic degeneration of hepatocytes. Circle areas show the slight necrosis of hepatocytes.

M-Zn and N-Zn groups presented the clinical histopathological changes as edema, hydropic degeneration, and slight necrosis of hepatocytes around the central vein. However, no difference between the N-Zn and the M-Zn mice for these changes was observed.

The renal clinical lesions of mice were shown as slight glomerulus swelling after treatment with nano- or micro-scale Zn (see Fig. 4). Moreover, the renal tubular dilatation and proteinaceous casts in tubules were only observed in the N-Zn treated mice.

Fatty degeneration in the cardiovascular cells was observed in heart tissues of the N-Zn treated mice (see Fig. 5). No significant clinical histopathological alteration of heart tissues was found in the M-Zn treated mice.

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

4. Discussion

The metal powder used in this study indicated a good scale homogeneity and purity. Hence, the toxic effects found by this study are unambiguously resulted from the particles themselves.

The severe symptoms as lethargy, nausea, vomiting, and diarrhea were presented in the mice treated with N-Zn powders at the beginning days, which were consistent with the previous reports of oral excess zinc salts (Lock and Janssen, 2003; Piao et al., 2003; Talcott, 2001; Chandra, 1984). However, only slight symptoms were found in the M-Zn treated mice. The above responses were in accordance with the observation of the body weight gain of the N-Zn and the M-Zn mice. The N-Zn mice exhibited significant growth retardation compared with both the M-Zn mice and the controls at the first three days after treatment. Therefore, the results indicated that the N-Zn oral exposure could cause more severe responses than the M-Zn treatment.

The two deaths from the N-Zn treatment indicated that nanoscale zinc powder was probably easier to cause intestinal obstruction. As nanoscale materials are easy to aggregate in various media, it is speculated that nanoscale Zn powder may easily aggregate in animal body as well. Thus, our results suggest that the N-Zn oral exposure could induce more severe intestinal response than the M-Zn.

The blood biochemical tests are frequently used in diagnosis diseases of heart, liver, kidney, and cardiovascular system, etc. They are also widely used in monitoring the response to the exogenous toxic exposure. The ALT is often tested along with AST, ALP, and LDH to evaluate whether the liver is damaged or diseased.

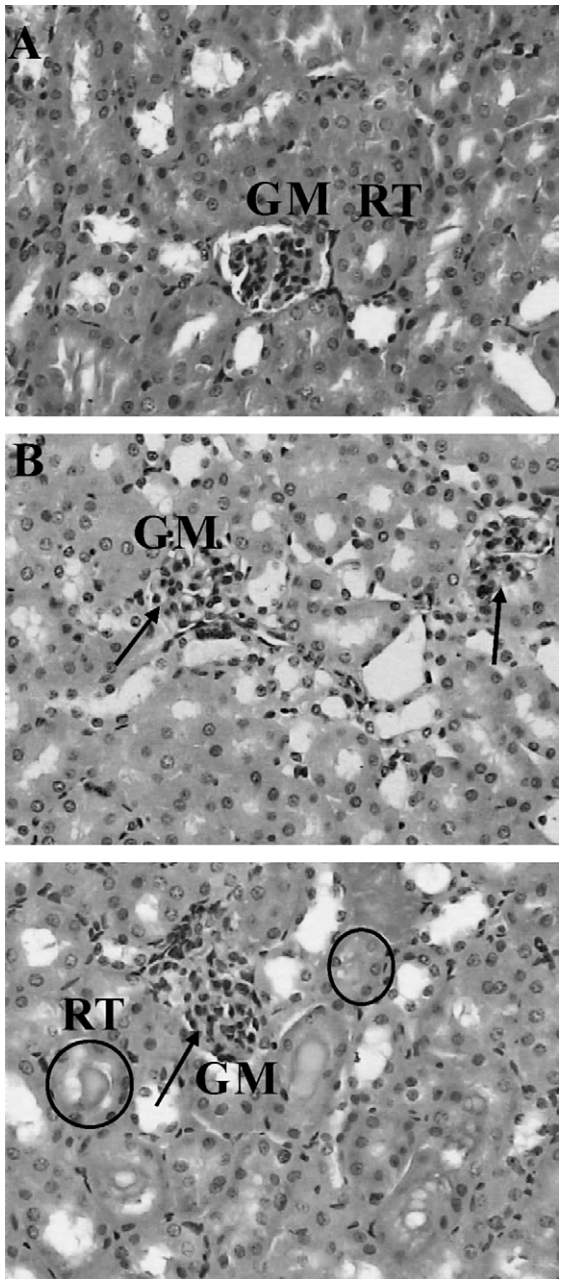

Fig. 4. Kidney tissue from mice exposed to zinc powder at an acute toxic dose of 5 g/kg body weight on 14 days post-oral administration (magnification = 200). (A) Control group (instilled 1% sodium carboxy methyl cellulose). GM: glomerulus; RT: renal tubular. (B) Microscale group. Arrows show the glomerulus swelling. (C) Nanoscale group. Arrows show the glomerulus swelling. Circle area show the proteinaceous casts in renal tubular.

When the liver is in dysfunction, the levels of the above enzymes will rise (Kellerman, 1995). Therefore, in this study the significantly elevated levels of ALT, AST, ALP, and LDH in the M-Zn mice, and ALT, ALP, and LDH

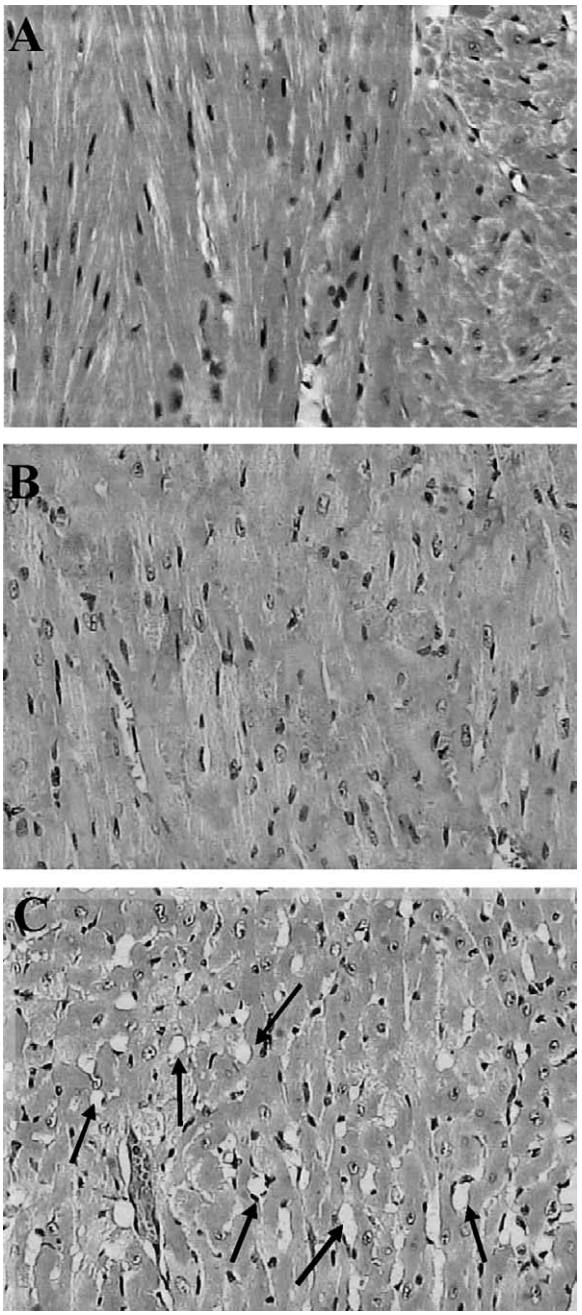

Fig. 5. Heart tissue from mice exposed to zinc powder at an acute toxic dose of 5 g/kg body weight on 14 days post-oral administration (magnification = 200). (A) Control group (instilled 1% sodium carboxy methyl cellulose). (B) Microscale group. (C) Nanoscale group. Arrows show the fatty degeneration in the cardiovascular cells.

in the N-Zn mice clearly indicated that the liver damage was induced by M-Zn and N-Zn. The higher levels lasted as long as two weeks. Additionally, the clinical changes were observed in the two treated group mice as well, which was corresponding with the results of blood measurements. Furthermore, compared with the responses of the N-Zn group mice, the higher levels of the enzymes were generally observed in the M-Zn mice, which implied that the M-Zn induced more severe liver damage than the N-Zn. Some previous studies also reported the liver damage by high zinc salt administration (Piao et al., 2003; Ding et al., 1998; Chen et al., 1992). Piao et al. (2003) found that intraperitoneal administration of high-dose zinc acetate could cause liver damage and decrease serum glutamic oxalacetic transaminase (GOT).

Jenkins and Kramer (1992) reported that cholesterol esters (CHE) and cardiolipin slightly increased after excess dietary zinc ingestion. Significantly higher CHE was also observed in the M-Zn mice of our study. Because CHE are hydrolyzed in liver by lysosomal cholesterol esterase (Stokke, 1974), therefore, the higher liver CHE concentration observed in previous and our studies indicated that excess Zn oral exposure might inhibit this esterase.

The blood BUN and CR are good indicators for renal function. If kidney function falls, the BUN and CR levels will rise. Thus, the significantly increased serum BUN and CR levels in the M-Zn mice in this study suggested that the renal dysfunction be most likely caused by M-Zn administration. Such result was also obtained from other researches of high zinc salts exposure. Llobet et al. (1988) reported that the concentrations of urea and CR in plasma significantly increased after high-dose exposure to zinc acetate dihydrate in drinking water. However, in this study, no such overt elevation was found in the N-Zn treated mice. But further renal histopathological examination revealed that there was alteration of proteinaceous casts in the tubules and renal tubular dilatation in the N-Zn treated mice, while only slight renal glomerulus swelling was found in the M-Zn treated mice. The histopathological finding demonstrated that the N-Zn oral exposure could cause more severe renal damage than the M-Zn, though the serum indicators did not show obvious changes. The BUN alteration is known to be caused by many factors besides renal disease, including protein breakdown, hydration, and liver failure (Kellerman, 1995). Therefore, in this research, the elevated BUN in the M-Zn mice could be partly caused by liver dysfunction.

The blood biochemical tests of CK, AST, LDH, and HBD are widely used as the conventional “cardiac enzymes” in hospital for diagnosis of heart diseases. Their elevated levels indicate the occurrence of the ischemic heart disease, acute coronary syndromes, etc. (Lee and Goldman, 1986). Our results showed that the activities of CK, AST, LDH, and HBD in the M-Zn

group, LDH and HBD in the N-Zn group obviously rose versus the controls. The abnormal alteration of HGB, HCT, RDW-CV, and PLT of both N-Zn and M-Zn mice illustrated that heavy anemia might occur in the mice after administration of high-dose zinc powders. The remarkably raised concentrations of PLT and RDW-CV and the obviously reduced levels of HCT and HGB in the N-Zn mice stated that the anemia was more severe in the N-Zn mice than in the M-Zn ones. Additionally, the histopathological examination of heart tissues in the N-Zn mice showed the fatty degeneration occurred in this organ, which could be caused by long-term anemia (Llobet et al., 1988).

Anemia induced by zinc and zinc compounds was reported by many other researchers (e.g. Llobet et al., 1988; Torrance and Fulton, 1987; Latimer et al., 1989; Hoffman et al., 1988) as well. Some of the previous studies concluded that excessive dietary zinc in animals could induce deficiencies of copper and iron and then produce growth retardation and anemia. The mechanism of anemia caused by the nanoscale zinc powder needs further study.

Recently, some important epidemiological studies reported that ultrafine particulates (air dynamic diameter < 100 nm) in the air had close relationship to mortality and morbidity of cardiovascular disease (Samet et al., 2000). One of the hypothesized mechanisms is that ultrafine particulates cause ischemic events involving enhanced clotting, haemostasis, and atheromatous plaque rupture, which increase blood coagulation and cause heart ischemia (Donaldson and Stone, 2003). Therefore, in this study the levels of PT, FIB, and APTT, which are related to blood coagulation in plasma, were measured, but no overt alteration was found in the two zinc treated mice.

5. Conclusions

In the experiment, the severe symptoms of lethargy, vomiting, and diarrhea were observed in the mice after an acute toxic dose oral administration of nanoscale zinc powders. The N-Zn mice exhibit significant growth retardation compared with the M-Zn and the control mice in the first three days after treatment. Two deaths caused by the N-Zn administration indicated that the nanoscale zinc was probably easily causing intestinal obstruction than the microscale zinc particles. Only slight differences of blood biochemical assay were found between micro- and nano-scale zinc powders. According to the literature and our findings, exposure to nano- and micro-scale zinc powders at high doses may produce toxic effects on the hematopoietic system, biochemical system and various tissues and organs, such as liver and kidney. Combined with the results of histopathological examination in this study, a preliminary conclusion could be drawn that the high-dose M-Zn oral exposure could induce more severe liver damage than N-Zn, while N-Zn could induce heavier renal damage and anemia.

However, our results do not mean that the nanoscale zinc powder is less or more toxic than the microscale one. The purpose of this study is just to evaluate the oral toxicity of nanoscale zinc powder according to the OCED guidelines that is currently used for testing toxicity of chemicals. Therefore, the dose used in this acute toxic study was higher than the real possible exposure level. Since nanoscale particles have large specific surface area, it is found that their biological effects are mainly dependent on their surface area rather than particle mass (Oberdörster et al., 1994). When comparing the health effects of chronically inhaled TiO_2 particles with different sizes, it was found that the low level exposure resulted in a greater lung tumour incidence than the high one (Heinrich et al., 1989). Additionally, the different exposure ways, such as inhalation, dermal contact, and different metal salts administration could cause different toxic effects. Therefore, further research upon different exposure routes, especially inhalation exposure, and long-term low level effect caused by nanoscale materials is now carried out in our laboratory.

Acknowledgements

The authors are grateful to the National Natural Science Foundation of China (10490180) and the Chinese Academy of Sciences (Grant No. KJCX2-N10).

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