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Synergistic toxicity of bulk zinc oxide and cadmium chloride in mice

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Abstract: The wide applications of zinc oxide (ZnO) and ubiquitous cadmium (Cd) pollution have increased the risk of humans' co-exposure to ZnO and Cd. The synergistic toxicity of bulk ZnO and $CdCl_2$ in mice was investigated. Mice were randomly divided into four groups: a control group and three experimental groups (bulk ZnO, $CdCl_2$, bulk ZnO+$CdCl_2$). Bulk ZnO shows low toxicity in mice. In contrast, $CdCl_2$ causes siginificant damage in the liver indicated by severe liver dysfunction and histopathological abnormalities. Although co-exposure to bulk ZnO and $CdCl_2$ has positive synergistic effects on the uptakes of Zn and Cd in the liver, bulk ZnO can significantly alleviate $CdCl_2$-induced damage in the liver. The bulk ZnO-induced metallothionein synthesis and the inhibition of Cd-induced deprivation of tissue Zn by bulk ZnO might play two key roles in the protective effect of bulk ZnO on $CdCl_2$-induced damage in the liver.

Key words: heavy metal; histopathology; synergistic toxicity; intraperitoneal injection

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氧化锌与氯化镉在老鼠体内的协同毒性

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摘要:随着氧化锌的广泛应用和镉的广泛污染,人类同时暴露于这两种污染物的概率大大增加.研究了氧化锌与氯化镉对小鼠的协同毒性.实验分为4组:①对照组;②氯化镉组;③氧化锌组;④同时注射氧化锌和氯化镉组.实验结果表明,氧化锌对小鼠毒性很小,相反,氯化镉对小鼠肝产生严重的功能和病理损伤.虽然小鼠在同时暴露于氧化锌和氯化镉时,锌和镉在肝中有正的协同聚集作用,但氧化锌能显著抑制氯化镉造成的肝损伤.氧化锌诱导的金属硫蛋白合成以及氧化锌对镉引起锌稳态失衡的抑制,在氧化锌抑制氯化镉毒性的作用中发挥重要作用.

关键词:重金属;组织病理学;协同毒性;腹腔注射

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0 Introduction

Zinc oxide (ZnO) has been widely used in diverse applications for hundreds of years, such as rubber[1], concrete[2], plastics[3-4], coatings[5], cosmetics[6] and so on. Generally, zinc oxide is categorized as a non-toxic material[7]. However, inhalation or ingestion of zinc oxide powder may be harmful to human health. It is known that Zn is involved in numerous aspects of cellular metabolism. It is required for the catalytic of many enzymes[8] and plays a role in immune function[9] and protein synthesis[10]. The widespread applications of ZnO in many aspects increase the potential for its release to the environment.

Cadmium (Cd) is a ubiquitous contaminant of the natural environment and dietary products[11-14] and has been recognized as a seriously hazardous substance[15] and a human carcinogenic[16]. The primary sources of Cd exposure are cigarette smoke, food intake and ambient air, particularly in the vicinity of battery, electroplating, pigment and plastic manufactories[17]. Acute exposure to Cd leads to accumulation of Cd mainly in the liver, while chronic exposure results in accumulation of Cd mainly in the liver and kidney. When the amount of Cd in the liver and kidney exceeds the binding capability of metallothionein (MT)[16,18-19] that has a high affinity to Cd, the non-MT-bound Cd can induce reactive oxygen species (ROS) that results in oxidative deterioration to lipids, proteins and DNA.

With the wide applications of ZnO and the ubiquitous Cd contamination, it is necessary to identify the synergistic effect of co-exposure to ZnO and Cd on human health. Several studies have examined the synergistic toxicity of Zn and Cd in vivo and in vitro, and their results have shown that Zn is able to protect against $CdCl_2$-induced toxicity. King et al.[20] reported that Zn plays a protective role in Cd-induced testicular toxicity in mice. Banni et al.[21] showed that Zn administration reduces Cd-induced toxicity in rats. To the best of our knowledge, earlier studies have focused on evaluating the synergistic toxicity between Cd and soluble Zn complexes, such as zinc acetate[20], $ZnCl_2$[21-22] and $ZnSO_4$[23]. The synergistic effect of co-exposure to insoluble zinc complex and Cd on animal and human health still remains unclear. Herein, we investigated the synergistic toxicity of bulk ZnO and $CdCl_2$ in mice. The biodistributions, serum biochemical parameters and histopathologic changes were determined to evaluate the synergistic toxicity of bulk ZnO and $CdCl_2$. Intraperitoneal injection, as one of the classic routes of administration[24-26], has been used in this study. The results indicate that like soluble Zn complexes, bulk ZnO can also reverse Cd-induced toxicity in mice.

1 Experimental

1.1 Animals and treatment

Bulk ZnO and cadmium chloride ($CdCl_2$) were purchased from Sigma Chemical Co. (St. Louis, USA). Bulk ZnO was characterized by transmission electron microscopy (TEM) (JEM-2010, JEOL Ltd., Japan). The particle size was determined to be (3±1)μm. Male Kun Ming mice (22 g±2, 6～8 weeks) were purchased from the Animal Center of Anhui Medical University (China). Animals were housed in stainless steel cages with filter tops under standard conditions (22 ℃±2; relative humidity at 55%±5%; 12 h light/dark cycle). Distilled water and sterilized food for mice were available ad libitum. All experiments were conducted in accordance with the guidelines of University of Science and Technology of China for the care and use of laboratory animals and with approval of the Animal Ethical Committee of University of Science and Technology of China. Mice were randomly divided into four groups with 10 male mice in each group: control group (treated with physiological saline) and three experimental groups (1.0 mg of $CdCl_2$ per kg of body weight, 20 mg of bulk ZnO per kg of body weight, 20 mg of bulk ZnO per kg of body weight+1.0 mg of $CdCl_2$ per kg of body weight). Chemicals were

administered to mice via intraperitoneal injection at a dose of 0.1 mL per 10 g of body weight, respectively, once a day for 7 consecutive days. For co-exposure, bulk ZnO was injected in mice 30 min before the administration of $CdCl_2$. The symptoms and mortality were observed and recorded carefully everyday. After 7 days, blood samples were collected from the eye vein by removing the eyeball quickly after being anaesthethetized by ether. Serum was harvested by centrifuging blood at 3 000 r/min for 15 min. The tissues and organs such as the heart, liver, spleen, lung, kidney and brain were excised, washed thoroughly with physiological saline and weighed. The coefficients of organs to body weight were calculated as the ratio of organs (wet weight, mg) to body weight (g).

1.2 Assay for serum biochemical parameters

Liver function was evaluated with serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and total bilirubin (TBIL). All biochemical parameters were determined by a biochemical autoanalyzer (Roche Modular DPP, Roche Ltd., Switzerland) using the commercial kits (Roche Ltd., Switzerland).

1.3 Histopathological examination

All histopathological examinations were performed using standard laboratory procedures. The tissues of the heart, liver, spleen, lung, kidney and brain were cut out and immediately fixed in 10% (volume fraction) formalin, embedded in paraffin blocks, then sliced into 5 μm in thickness and placed onto glass slides. After hematoxylin-eosin staining, the slides were observed and the photos were taken using an optical microscope (IX-81, Olympus, Japan) at 40 × magnification. The identity and analysis of the pathology slides were blind to the pathologist.

1.4 Analysis of the uptakes Zn and Cd in liver

The liver tissues (0.1～0.3 g) were weighed and digested with ultrapure nitric acid overnight, respectively. Then the solutions were heated at 120 ℃ to remove the remaining nitric acid until they were colorless and clear. Finally, the remaining solutions were diluted to 5 mL with 2% nitric acid. The Zn and Cd concentrations in the solutions were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Perkin Elmer Corporation, USA). Data were expressed as micrograms per gram fresh tissue.

1.5 Statistical analysis

Results were expressed as means ± SD (n=10). Data were analyzed by one-way analysis of variance (ANOVA) test using SPSS 16.0 (SPSS Inc., Chicago, IL). The Dunnett's test was used to compare the differences between the experimental groups and the control group. The statistical significance for all tests was set at $p<0.05$.

2 Results and discussion

2.1 Effects of Co-exposure on coefficients of organs

During the entire exposure period, the daily behaviors such as eating, drinking and activity in all exposed groups were as normal as the control group. The body weights of the mice in throughout exposure period are shown in Fig. 1. There was no significant difference between the control group and bulk ZnO group, whereas the body weight in the $CdCl_2$ group increased more slowly than the control group. Interestingly, the body weight in the bulk ZnO + $CdCl_2$ group increased faster than the $CdCl_2$ group. These results suggest that $CdCl_2$ (1.0 mg per kg of body

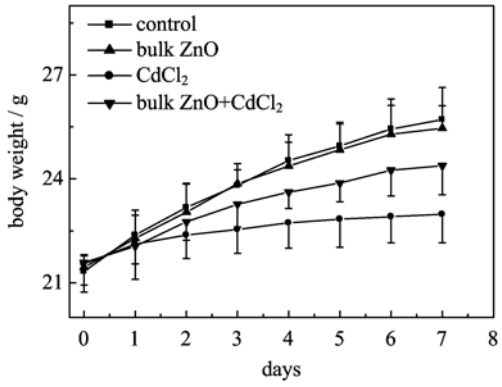
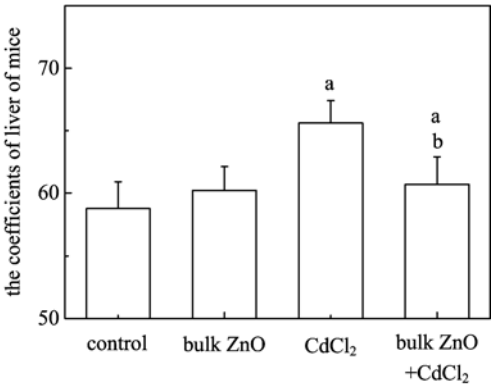


Fig. 1 Body weight increases of mice (means±SD, n=10) during exposure period

weight) obviously slows down the growth of the mice and bulk ZnO (20 mg per kg of body weight) significantly alleviates the toxic effect of $CdCl_2$ on the mice.

As shown in Fig. 2, there were no statistically significant differences between the control and bulk ZnO groups in the coefficients of liver ($p>0.05$). In the $CdCl_2$ groups, the liver showed significantly higher coefficients than the control ($p<0.05$). Interestingly, the animals' co-exposure to bulk ZnO and $CdCl_2$ showed a significantly decrease in the coefficient of the liver compared to the $CdCl_2$ group ($p<0.05$), suggesting that bulk ZnO markedly reduces the toxic effect of $CdCl_2$ on the liver.

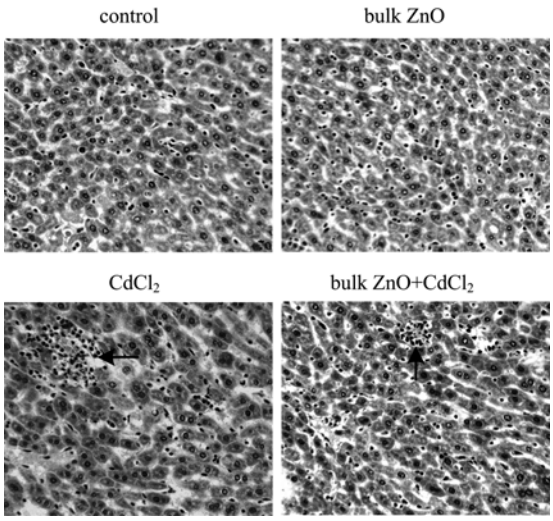


The mice were intraperitoneally injected with bulk ZnO and/or $CdCl_2$ for seven consecutive days.
Data were expressed as means±SD ($n=10$).
a: $p<0.05$ versus the control;
b: $p<0.05$ versus the $CdCl_2$ group.

Fig. 2　The coefficients of liver of mice

2.2　Effects of Co-exposure on histopathological changes in tissues

Fig. 3 shows the histopathological photomicrographs of the liver tissues. In the control group, hepatocytes were integrated and well arranged. In the bulk ZnO groups, no obvious abnormality was observed. In the $CdCl_2$ group, the severe spotty necrosis was observed in the liver tissue, revealing $CdCl_2$-induced severe damage in liver tissue. The $CdCl_2$-induced histopathological damage in the liver resulted in a significant increase in the coefficient of the liver (Tab. 1). Interestingly, in the bulk ZnO + $CdCl_2$ group, the spotty necrosis in small areas was found in the liver tissue. These results indicate that bulk ZnO significantly attenuates $CdCl_2$-induced damage in the liver.



In the control group, hepatocytes were integrated and well arranged.
In the bulk ZnO group, no obvious abnormality was observed.
In the $CdCl_2$ group, the spotty necrosis (arrows) was observed.
In the bulk ZnO+$CdCl_2$ group, the slight necrosis (arrows) was induced

Fig. 3　Histopathological sections of the liver tissue in mice following daily intraperitoneal injections of bulk ZnO and/or $CdCl_2$ for 7 days

2.3　Effects of Co-exposure on serum biochemical parameters

To further investigate the protective role of bulk ZnO in Cd^{2+} toxicity, the serum biochemical

Tab. 1　Changes of biochemical parameters in serum of mice

Indexes	Control	bulk ZnO	$CdCl_2$	bulk ZnO+$CdCl_2$
ALT/(U·L^{-1})	53.5±2.1	69±2	152±4^a	67±2^b
AST/(U·L^{-1})	111±2	136±3	184±5^a	116±4^b
ALP/(U·L^{-1})	120±4	116±2	157±4^a	121±2^b
LDH/(U·L^{-1})	867±6	1095±9^a	1319±18^a	945±7^b
TBIL/(μmol·L^{-1})	1.03±0.10	1.15±0.07	1.42±0.22^a	1.05±0.11^b

【Note】 The mice were intraperitoneally injected with bulk ZnO and/or $CdCl_2$ for seven consecutive days. Data were expressed as means±SD ($n=10$). a: $p<0.05$ versus the control; b: $p<0.05$ versus the $CdCl_2$ group.

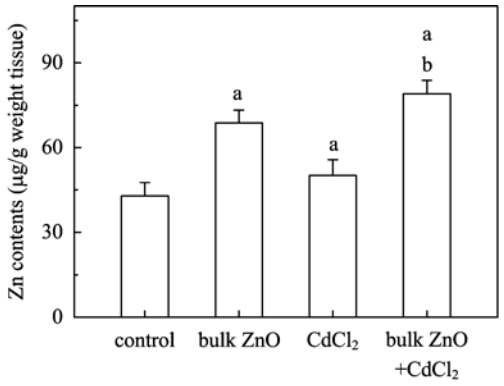
parameters in the mice after exposure to bulk ZnO and/or $CdCl_2$ were determined and shown in Tab. 1. In the bulk ZnO group, there were no significant changes in all serum biochemical parameters with the control group, except that the LDH level showed a significantly increased level ($p<0.05$), suggesting that exposure to bulk ZnO only slightly affects the liver functions. By contrast, the mice's exposure to $CdCl_2$ showed significant changes in all tested serum biochemical parameters of liver function compared to the control group. The significant increases in ALT, AST, ALP, LDH and TBIL levels ($p<0.05$) indicated the severe liver dysfunction caused by $CdCl_2$, which was in agreement with the findings of Abdel-Aziem et al.[27] who found that Cd causes marked alterations in the liver functions of mice. LDH is a stable cytoplasmic enzyme that widely exists in all cells. LDH is released into the blood serum when the cell membrane is damaged. The significant increase in LDH activity should be attributed to hepatocellular necrosis. These observations together with the histopathology results further confirmed severe liver injury in the $CdCl_2$ group.

Interestingly, in the bulk ZnO+$CdCl_2$ group, the levels of hepatic biochemical parameters ALT, AST, LDH and TBIL were significantly different from those in the $CdCl_2$ groups, but very close to those in the bulk ZnO group, suggesting that co-exposure to bulk ZnO and $CdCl_2$ only slightly affects the liver function, which is similar to in the bulk ZnO group. These data together with the histopathology results confirm that bulk ZnO significantly attenuates $CdCl_2$-induced damage in the liver.

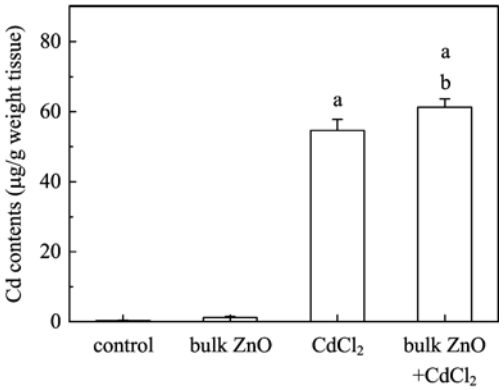
2.4 Effects of co-exposure on the uptakes of Zn and Cd in liver

The uptakes of Zn and Cd in liver were studied by ICP-AES after exposure to bulk ZnO and/or $CdCl_2$. In the bulk ZnO group, the Zn level was significantly higher than that of the control group ($p<0.05$). In the $CdCl_2$ group, the Cd and Zn levels were significantly higher than those of the control group ($p<0.05$). The higher accumulation of Cd and Zn in the liver after exposure to $CdCl_2$ is mainly due to a low-molecular-weight protein, metallothionein (MT), which shows a high binding affinity to Cd^{2+} and Zn^{2+}[28]. The liver contains high amounts of MT, which contributes to the high Cd and Zn levels in the liver[18]. In bulk ZnO+$CdCl_2$ group, the Zn level was significantly higher than that of either $CdCl_2$ or bulk ZnO group; on the other hand, the Cd level was also significantly higher than that of the $CdCl_2$ group ($p<0.05$) (Fig. 4(b)). These results reveal that co-exposure to bulk ZnO and $CdCl_2$ has a positive cooperative effect on the uptakes of Zn and Cd in the liver. The results are consistent with the previous findings. King et al.[20] reported that exposure to zinc acetate by subcutaneous injection significantly increased the Cd content of the liver in mice. Oishi et al.[29] found that exposure to $CdCl_2$ by oral or intravenous administration increased Zn level in the liver of rats. Both Zn[22] and Cd[29] have been reported to induce MT synthesis in the liver, which contributes to the positive synergistic uptake of Zn and Cd in the liver.

Although bulk ZnO enhances Cd uptake in the liver, it significantly reduces the Cd-induced toxicity in the liver. The protective effect of bulk ZnO against $CdCl_2$-induced liver damage may be attributed to the bulk ZnO-induced MT synthesis in the liver. MT protects cells from Cd toxicity, because it sequesters Cd and reduces the amount of Cd available to exert toxic actions. On the other hand, Cd toxicity was reported to be attributed to a metabolic disorder in some trace elements, mainly Zn[21]. Many enzymes contain Zn^{2+} in their active sites. Zn^{2+} in these enzymes can be replaced by Cd^{2+}, thereby making the enzymes inactive[30]. Co-exposure to bulk ZnO and $CdCl_2$ increased Zn content in the liver, and thereby inhibited the displacement of Zn by Cd through the competitive interaction between Zn and Cd, and reduced the



(a) The contents of Zn in liver tissue of mice



(b) The contents of Cd in liver tissue of mice

The mice were intraperitoneally injected with bulk ZnO and/or $CdCl_2$ for 7 days.

a: $p<0.05$ versus the control;

b: $p<0.05$ versus the $CdCl_2$ group.

Values represent means±SD, $n=10$

Fig. 4　Biodistributions of Zn and Cd in liver of mice determined by ICP-AES

perturbation of Zn homeostasis. It is interesting to note that although the high Cd accumulation in the liver of the mice occurred after co-exposure to bulk ZnO and $CdCl_2$, the liver dysfunction and damage were still very slight, which suggests that the increase in Zn uptake after co-exposure to bulk ZnO and $CdCl_2$ should take another important role in the bulk ZnO-mediated protection of Cd toxicity.

3　Conclusion

In summary, we report that like soluble Zn complexes, bulk ZnO and $CdCl_2$ have a negative synergistic toxicity in mice. Although co-exposure to bulk ZnO and $CdCl_2$ has positive synergistic effects on the uptakes of Zn and Cd in the liver, bulk ZnO can significantly alleviate $CdCl_2$-induced damage in the liver, as indicated by slight changes in liver function and histopathology. The bulk ZnO-induced MT synthesis and the inhibition of Cd-induced deprivation of tissue Zn by bulk ZnO may play two key roles in the protective effect of bulk ZnO on $CdCl_2$-induced damage in the liver.

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