Protective effect of Fe$_3$O$_4$ nanoparticles on cadmium chloride-induced toxicity in the small intestine of mice

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Abstract：Synergistic toxicity from multiple environmental pollutants poses greater threat to humans. Here we evaluated combined toxicity of environment pollutants Fe$_3$O$_4$ nanoparticles (nano-Fe$_3$O$_4$) and cadmium chloride (CdCl$_2$) in the small intestine of mice. The results showed that Fe$_3$O$_4$ nanoparticles (nano-Fe$_3$O$_4$) and CdCl$_2$ have a negative synergistic toxicity in the small intestine of mice. Oral nano-Fe$_3$O$_4$ did not show obvious toxicity in the small intestine of mice. In contrast, oral CdCl$_2$ caused significant oxidative stress in the small intestine of mice. CdCl$_2$-induced oxidative stress resulted in inflammatory response in the small intestine as indicated by the significant increases in the levels of cyclooxygenase-2 and nitric oxide synthase as well as the inflammatory cell infiltration in the small intestinal tissue. Co-exposure to nano-Fe$_3$O$_4$ and CdCl$_2$ significantly attenuated the CdCl$_2$-induced damage in the small intestine through reduction of oxidative stress and inflammatory response.

Key words：nano-Fe$_3$O$_4$; Cd; synergistic toxicity; small intestine

纳米四氧化三铁对氯化镉诱导小鼠小肠毒性的保护作用

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摘要：环境中多种污染物的协同毒性对人类健康有很大威胁. 本文报道了环境中污染物纳米四氧化三铁和氯化镉对小鼠小肠的协同毒性. 结果显示，纳米四氧化三铁和氯化镉对小鼠小肠有负协同毒性. 口服纳米四氧化三铁对小鼠小肠没有明显的毒性. 相反，口服氯化镉引起小鼠小肠的氧化应激反应，该氧化应激进一步导致小鼠小肠炎症反应，表现为小肠组织中两个炎症指标(环氧合酶和一氧化氮合成酶)水平显著升高和小肠组织出现炎细胞浸润. 同时口服纳米四氧化三铁与氯化镉后，纳米四氧化三铁能够显著降低由氯化镉诱导的小肠的氧化应激反应，从而显著减少氯化镉对小肠的损伤. 纳米四氧化三铁和氯化镉在小肠组织中相互竞争禁阻摄取，导致纳米四氧化三铁和氯化镉对小肠中铁和镉的含量有负的协同作用. 纳米四氧化三铁不仅能显著减少镉在小肠中积累，而且能抑制由镉引起的小肠中铁的缺乏，这是纳米四氧化三铁保护小肠免遭氯化镉引起的氧化损伤的两个关键作用机制. 因此，纳米四氧化三铁可以作为由镉引起肠道损伤患者的口服磁共振造影剂和药物载体.

关键词：纳米四氧化三铁；镉；联合毒性；小肠

0　Introduction

Superparamagnetic iron oxide nanoparticles (SPION) with good magnetic responsiveness, biocompatibility and low toxicity have been extensively used in diverse applications such as hyperthermia, contrast agents for magnetic resonance imaging and drug delivery[1-2]. Among a large number of metal oxide nanoparticles, only SPION is the approved magnetic nanomaterial for

the clinical use by the US Food and Drug Administration[3]. In addition, SPION has been widely used in consumer products and industrial applications[4-7], and consequently, their release into and eventual accumulation in the environment continue to grow exponentially. As a result, the concentration of iron oxides was found to be as high as 12.8 to 44 ng/L in Europe[8]. With the wide applications of SPION, its potential risk to the health of human is increasing[9-11]. Previous studies revealed that the SPION has low toxicity in vivo and in vitro[12].

Cadmium (Cd) is a ubiquitous pollutant of the dietary products and natural environment[13-14], which has been listed as one of the Top Twenty Hazardous Substances Priority by the Agency for Toxic Substances and Disease Registry[15-16]. The reported Cd concentration in industrial wastewater is high as 3707 μg/L in Saudi Arabia[17] and 970 μg/L in India[18]. The Cd concentration in surface water is as high as 14 μg/L in Singapore[19]. Oral exposures to Cd^{2+} cause injuries in respiratory system, liver, kidney, skeleton, reproductive system, and even cause cancer[[20].

With the ubiquitous Cd pollution and the wide application of SPION, it is *of great necessity* to investigate the combined toxicity of SPION and Cd in humans and animals. Our recent studies revealed that Fe_3O_4 nanoparticles (nano-Fe_3O_4) markedly alleviated $CdCl_2$-induced injury in the kidney and liver of mice[21]. However, the combined toxicity of nano-Fe_3O_4 and $CdCl_2$ in the gastrointestinal tract of mice is still unclear. As therapeutic agents, SPIONs were most commonly administrated by oral delivery[22-23]. SPION is suitable for oral delivery as the MRI contrast agent for the gastrointestinal tract organ[24]. Currently, we are not assured that whether SPIONs are suitable candidates as oral delivery of MRI contrast agents for patients with the Cd-induced diseases of the gastrointestinal tract organs.

In this study, we investigated the combined acute toxicity of nano-Fe_3O_4 and $CdCl_2$ in the small intestine of mice. This investigation is crucial for the applications of SPION in clinical diagnosis and therapy of Cd poisoning. The result reveals that co-administration of $CdCl_2$ with nano-Fe_3O_4 has a negative synergistic effect on the biodistributions of Fe and Cd in the small intestine. Nano-Fe_3O_4 can prevent the oxidative damage of the small intestine induced by $CdCl_2$.

1　Experimental

1.1　Chemicals

Commercially available nano-Fe_3O_4 was purchased from Alfa Aesar (Ward Hill, MA, USA). The JEM-2010 transmission electron microscopy (JEOL Ltd, Japan) was used to characterize nano-Fe_3O_4. $CdCl_2$ was obtained from Sigma Chemical Co. (St. Louis, USA).

1.2　Materials

Male Kunming mice (23 g $\pm$ 2 g, 7 weeks) were obtained from Animal Center of Anhui Medical University (China). All mice were raised under standard conditions[25]. All animal experiments were carried out in accordance with the guideline[16] of USTC for the use of animals. Mice were randomly assigned to four groups: control group (saline solution), nano-Fe_3O_4 group (50 mg/kg body weight (BW)), $CdCl_2$ group (2.0 mg/kg BW), and nano-Fe_3O_4 + $CdCl_2$ group (50 mg/kg BW nano-Fe_3O_4 + 2.0 mg/kg BW $CdCl_2$ group) (10 male mice per group). The chemicals were intragastrically injected into mice for seven days. After 7 days, all mice were sacrificed after anaesthetization with an intraperitoneal injection of 20% urethane solution (5 mL/kg BW). The small intestine was dissected out. The ratio of the wet weight (mg) of the small intestine to body weight (g) was calculated as the small intestine coefficient. Throughout the experimental period, the mice were observed daily for the health status.

1.3　Measurement of contents of Cd and Fe in the small intestine

Following the previous method[26], an OPTIMA 7300DV inductively coupled plasma-atomic emission spectrometry (PerkinElmer, USA) (ICP-AES) was used to detect the contents of Cd and Fe in the small intestine.

1.4　Histopathological examinations

An IX-81 optical microscope (Olympus, Japan) was used to analyze the histopathological sections of the small intestine tissues following the previous method[26].

1.5　Analysis of oxidative stress

The activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) and the level of malondialdehyde (MDA) were determined by the previous methods[25].

1.6　Analysis of cytokine expressions

The levels of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in the tissues of the small intestine were measured by ELISA using commercial kits (Shanghai Jianglai Biotechnology Co., *Ltd*., Shanghai, China), following the

manufacturer's instructions.

1.7　Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the data. The significance of differences was set at $p < 0.05$ for all tests. Data were shown as the mean value $\pm$ SD ($n = 10$).

2　Results

2.1　Characterization of nanoparticles

TEM was used to characterize the nano-Fe₃O₄ particle size. Fig. 1 shows that the particles were uniform in sizes and well-dispersed and had an average size of 14.1 nm $\pm$ 2.6 nm.
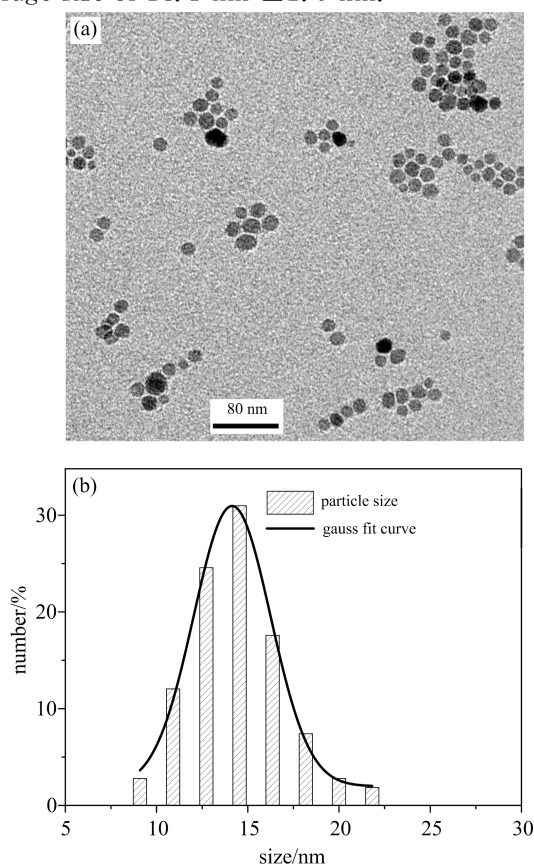

Fig. 1　TEM image of nano-Fe₃O₄ with a mean size of 14.1 nm $\pm$ 2.6 nm. (a) Representative TEM image and (b) size distribution profile.

2.2　Effects of co-exposure on the coefficient of the small intestine

During the entire exposure period, no abnormal daily activity and symptoms were observed in all groups, except for slight diarrhea in the mice in the CdCl₂ group. Fig. 2 shows that oral nano-Fe₃O₄ did not influence the small intestine coefficient significantly compared to the control. In the CdCl₂ group, the coefficient of the small intestine was much higher than the control ($p < 0.05$), suggesting the CdCl₂-induced injury in the organ. Interestingly, co-administration of nano-

Fe₃O₄ with CdCl₂ caused a significant decrease in the small intestine coefficient compared to the CdCl₂ group ($p < 0.05$), revealing that nano-Fe₃O₄ greatly attenuated the toxicity of CdCl₂ in the small intestine.
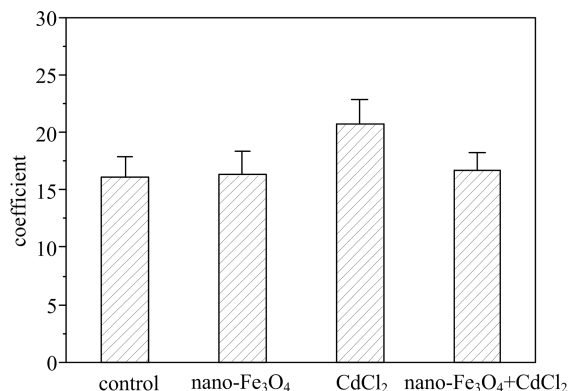

Fig. 2　The small intestine coefficient of the mice after intragastrical injection of CdCl₂ and/or nano-Fe₃O₄ for 7 days. Data were displayed as mean $\pm$ SD, $n = 10$. $^*p < 0.05$ vs. the control group.

2.3　Effects of co-exposure on biodistributions of Fe and Cd in the small intestine

Fig. 3 showed the contents of Fe and Cd in the small intestine of mice. Oral nano-Fe₃O₄ significantly increased the content of Fe in mouse small intestine. However, oral CdCl₂ decreased the content of Fe in the small intestine of the mouse. Interestingly, the Fe content in the small intestine of the mouse of the nano-Fe₃O₄ + CdCl₂ group was much lower than that in the nano-Fe₃O₄ group ($p < 0.05$). The Cd contents were beyond the detection limit of ICP-AES in the nano-Fe₃O₄ group and the control group. In the CdCl₂ group, Cd content increased a lot in the small intestine. The Cd content in the small intestine in the nano-Fe₃O₄ + CdCl₂ group was much lower than the CdCl₂ group ($p < 0.05$). The results suggested that co-administration of CdCl₂ with nano-Fe₃O₄ had a negative cooperative influence on the uptake of Cd and Fe in the small intestine of the mouse.

2.4　Effects of co-exposure on histopathological changes in small intestinal tissue

Fig. 4 shows the histopathological photomicrographs of the small intestinal tissues. No pathology changes were observed in the tissues of the small intestine in the nano-Fe₃O₄ group and the control group. However, the inflammatory cell infiltration (ellipse) and the erosion and partial loss of the intestinal glands (arrows) were observed in the small intestinal tissue in the CdCl₂ group. The serious histopathological damages in the small intestine after the CdCl₂ treatment might be correlated with the high accumulation of Cd in
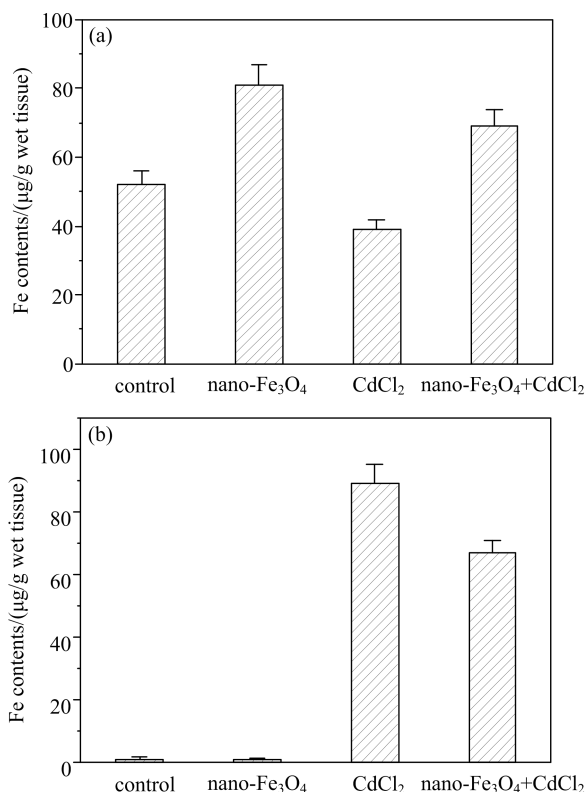
Fig. 3 The Fe content (a) and Cd content (b) in mouse small intestine are determined by ICP-AES after intragastrical injection of CdCl₂ and/or nano-Fe₃O₄ for 7 days. Data were displayed as mean $\pm$ SD, $n = 10$. * $p < 0.05$ vs. the control group.
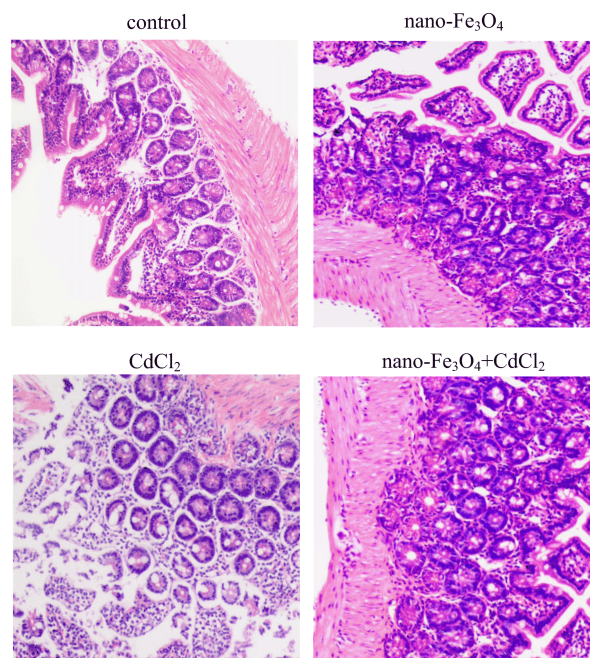

Fig. 4 Histopathologic section of the tissue of mouse small intestine after intragastrical injection of CdCl₂ and/or nano-Fe₃O₄ for 7 days. (a) the normal tissue of small intestine of control group; (b) the normal tissue of small intestine in the nano-Fe₃O₄ group; (b) the CdCl₂ group displays the inflammatory cell infiltration (ellipse) and the erosion and partial loss of the intestinal glands (arrows); (d) the nano-Fe₃O₄ + CdCl₂ group shows normal small intestine tissue.

the organ. Interestingly, after co-exposure to nano-Fe₃O₄ and CdCl₂, no obvious inflammatory response and injury were observed in the small intestinal tissue, indicating that nano-Fe₃O₄ markedly reduced the toxic effect of CdCl₂ on the small intestine.

2.5 Nano-Fe₃O₄ reduced CdCl₂-induced oxidative stress

Oxidative stress in the small intestine was detected after 7 days' oral administration of nano-Fe₃O₄ and/or CdCl₂. Fig. 5((a) and (b)) shows that oral nano-Fe₃O₄ had no significant effect on the activities of GPx and SOD, indicating that no observable oxidative stress was induced by oral nano-Fe₃O₄ in the small intestine of the mouse. However, oral CdCl₂ resulted in marked decreases in the activities of GPx and SOD, revealing that an oxidative stress was induced by oral CdCl₂ in the small intestine of the mouse. Interestingly, compared with the CdCl₂ group, the activities of GPx and SOD remarkably increased after co-administration of CdCl₂ with nano-Fe₃O₄, suggesting that the CdCl₂-induced oxidative stress in the small intestine was considerably reduced by nano-Fe₃O₄.

The lipid peroxidation product (MDA) is one main manifestation of oxidative damage. Fig. 5(c) shows that oral nano-Fe₃O₄ had no effect on the MDA level, indicating that oral nano-Fe₃O₄ didn't result in an observable oxidative stress in the small intestine of the mouse. However, the MDA level increased drastically in the small intestine caused by oral CdCl₂, indicating that CdCl₂ induced a lipid peroxidation in small intestine of the mouse. Compared with the CdCl₂ group, the MDA level decreased sharply after co-administration of nano-Fe₃O₄ with CdCl₂, revealing that the CdCl₂-induced oxidative stress in the small intestine was markedly reduced by nano-Fe₃O₄.

2.6 Nano-Fe₃O₄ reduced CdCl₂-induced inflammatory response

To analyze the nano-Fe₃O₄ and CdCl₂-induced inflammatory response, ELISA was used to examine the levels of COX-2 and iNOS. Fig. 6 shows that the COX-2 and iNOS levels in the small intestine of the mouse did not change after oral administration of nano-Fe₃O₄ ($p > 0.05$), suggesting that oral nano-Fe₃O₄ didn't induce inflammatory response in the small intestine. However, oral CdCl₂ resulted in marked increases in the iNOS and COX-2 levels in the small intestine, suggesting CdCl₂-induced the inflammatory response in the small intestine. Compared with the CdCl₂ group, the levels of
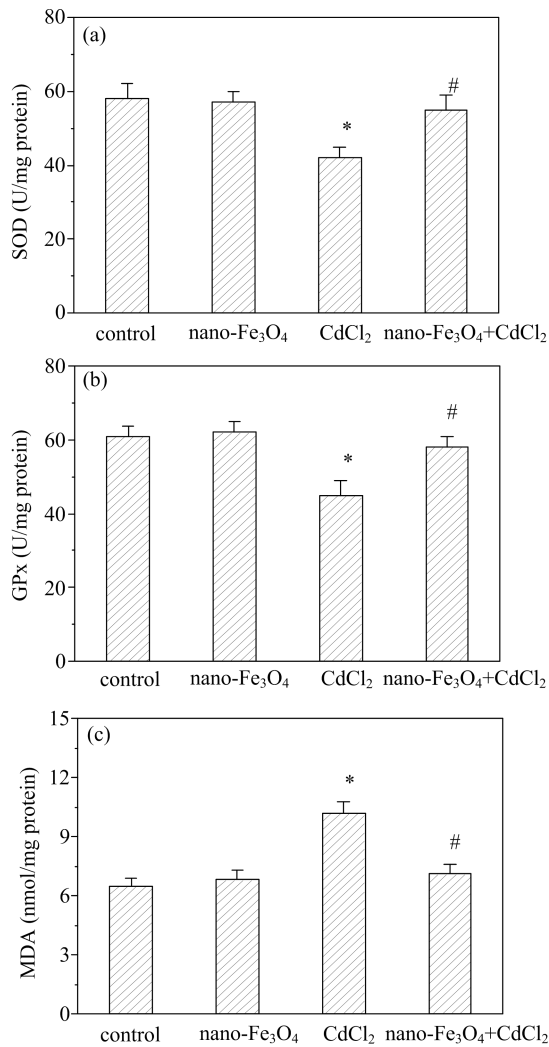
Fig. 5 The level of MDA and the activities of GPx and SOD in mouse small intestine after intragastrical injection of CdCl₂ and/or nano-Fe₃O₄ for 7 days. Data were displayed as mean ± SD，$n = 10$．* $p < 0.05$ vs. the control group；# $p < 0.05$ vs. the CdCl₂ group.

COX-2 and iNOS significantly decreased after co-administration of CdCl₂ with nano-Fe₃O₄, suggesting that CdCl₂-induced the inflammatory response in the small intestine was greatly reduced by nano-Fe₃O₄.

3 Discussion

  In the present study，the combined toxicity of co-exposure to nano-Fe₃O₄ and CdCl₂ in the small intestine of mice was determined. We demonstrated that co-exposure to CdCl₂ and nano-Fe₃O₄ has a negative synergistic effect on the biodistribution of Fe and Cd in the small intestine. Cd in the small intestine is taken up by a Fe²⁺ transporter，divalent metal transporter 1 (DMT1)[27]．DMT1 transports both Fe²⁺ and Cd²⁺．CdCl₂ and nano-Fe₃O₄ have negative cooperative effect on the biodistribution of Cd and
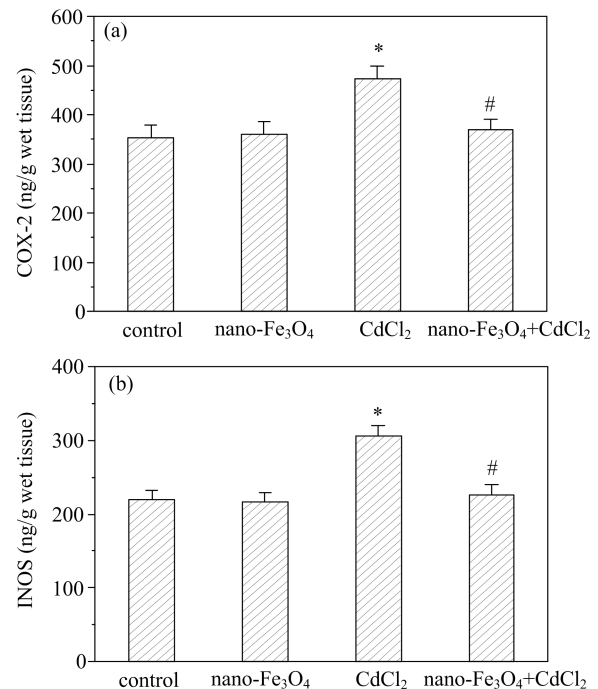

Fig. 6 Changes in COX-2 and iNOS levels in mouse small intestine after intragastrical injection of CdCl₂ and/or nano-Fe₃O₄ for 7 days. Data were displayed as mean ± SD，$n = 10$．* $p < 0.05$ vs. the control group；# $p < 0.05$ vs. the CdCl₂ group.

Fe in the small intestine of the mouse，suggesting that Cd and Fe competitively inhibit the uptake of each other in that both share the same uptake pathway through DMT1. This result is in agreement with the other report，which indicates that Cd²⁺ and Fe²⁺ inhibit competitively the uptake of each other in the intestinal epithelial cells of fish[28].

  The histopathology results indicated that nano-Fe₃O₄ markedly protects against the CdCl₂-induced injury in the small intestine，which may be partially caused by the nano-Fe₃O₄-induced decrease in Cd accumulation in the organ. It is notable that although the Cd accumulation in the small intestine of the mice，co-exposure to nano-Fe₃O₄ and CdCl₂ was still much higher than the control group co-exposure to nano-Fe₃O₄ and CdCl₂ did not induce obvious pathological changes in the small intestine，suggesting that besides the reduction of Cd accumulation in the small intestine by nano-Fe₃O₄，another mechanism may be also responsible for the protective role of nano-Fe₃O₄. Fig. 3(a) shows that exposure to CdCl₂ markedly decreased the level of Fe in mouse small intestine. Cd not only replaced Fe in Fe-dependent protein and enzyme but also inhibited Fe uptake in mouse small intestine[29]，which resulted in Fe deficiency as well as disturbed the Fe metabolism. Several reports indicate that the major feature of Cd

toxicity in mice is Cd-induced Fe deficiency[29-31]. Co-exposure to nano-Fe_3O_4 and $CdCl_2$ causes a dramatic increase in Fe level in mouse small intestine, the Fe level in which is higher than that of either the $CdCl_2$ group or the control group (Fig. 3(a)). The reduction of the Fe substituent in Fe-dependent protein by Cd and the increment of Fe uptake after co-exposure might be another key mechanism for the protection of Cd toxicity mediated by nano-Fe_3O_4.

Exposure to nano-Fe_3O_4 did not cause the oxidative stress in the small intestine of mice. However, exposure to $CdCl_2$ caused the significant oxidative stress in the organ. The oxidative stress could be attributed to the high accumulation of Cd in the small intestine after exposure to $CdCl_2$. SOD contains Zn and Cu in its active site. Zn in SOD can be replaced by Cd, thereby making the enzyme inactive[32]. The substitution of Zn in SOD by Cd might be the reason for the reduction of SOD activity induced by Cd. There is Se-Cys residue at the active site of GPx. Cd can interact with Se to form a complex at the active site and disrupts GPx activity[33]. The decrease in GPx activity should be caused by the interaction of Cd with the Se in GPx. $CdCl_2$-induced oxidative stress caused lipid peroxidation in the small intestine as indicated by the significant increase in its MDA level. Compared with the $CdCl_2$ group, the GPx and SOD activities apparently increased after co-administration of $CdCl_2$ with nano-Fe_3O_4, which was caused by nano-Fe_3O_4-induced reduction of Cd accumulation in the small intestine of the mouse.

Oral nano-Fe_3O_4 did not induce the inflammatory response in the small intestine. Oral $CdCl_2$ resulted in significant increases in the levels of COX-2 and iNOS in the small intestinal tissue. COX-2 performs a key role in the inflammation process, and the overexpression of COX-2 is an inflammation marker[34]. Between oxidative stress and inflammation, COX-2 serves as a bridging molecule. INOS is a rate-limitation enzyme which is responsible for NO synthesis. It has been shown that the increase of iNOS expression results in the production of high concentration of NO, which stimulates lipid oxidation[35]. The overexpression of COX-2 and iNOS in the small intestine reveals that the oral $CdCl_2$ induces the inflammatory response in the small intestine, which is further confirmed by pathological examinations.

The $CdCl_2$-induced oxidative damage in the small intestine could be reduced by nano-Fe_3O_4 through the following mechanisms. First, Fe was widely found at the active site of many proteins and enzymes the functions of which are critical for life[36]. The Fe in these proteins and enzymes

could be replaced by Cd and exposure to Cd could perturb the Fe homeostasis. Co-administration of nano-Fe_3O_4 with $CdCl_2$ increased Fe content in the mice, and thereby inhibited the displacement of Fe by Cd through the competitive interaction between Fe and Cd, and reduced the perturbation of Fe homeostasis. Second, the decrease in Cd accumulation in the mice after co-administration of nano-Fe_3O_4 with $CdCl_2$ could reduce the displacement of Zn in SOD by Cd and therefore increased the SOD activity. Third, the decreases in Cd accumulation in the mice after co-administration of nano-Fe_3O_4 with $CdCl_2$ could reduce the $CdCl_2$-induced toxicity in the small intestine.

4 Conclusion

In summary, nano-Fe_3O_4 and $CdCl_2$ have a negative synergistic acute toxicity in the small intestine of mice. Co-exposure to nano-Fe_3O_4 and $CdCl_2$ significantly attenuates $CdCl_2$-induced damage in the small intestine, which is attributed to the negative cooperative influence of co-administration of $CdCl_2$ with nano-Fe_3O_4 on the biodistribution of Cd and Fe in the small intestine of the mouse. Cd and Fe inhibit competitively the uptake of each other. The reduction of the accumulation of Cd in the small intestine and inhibition of Cd-induced deprivation of Fe in the tissue after co-exposure to $CdCl_2$ and nano-Fe_3O_4 may play two pivoyal roles in the protective influence of nano-Fe_3O_4 on the $CdCl_2$-induced injury in the small intestine. Nano-Fe_3O_4 may be used as a potential MRI contrast agent and drug carrier for patients with the Cd-induced diseases of the gastrointestinal tract organs in that it not only works well as a drug carrier or MRI contrast agent but also alleviates Cd toxicity simultaneously.

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