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Toxic effect comparison of three typical sterilization nanoparticles on oxidative stress and immune inflammation response in rats

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Abstract

Zinc oxide, titanium dioxide and silver nanoparticles are used as sterilization materials to enhance the performance of disinfectants. Here, the toxicological effects on the liver, spleen, thymus gland, immune function and inflammatory responses in rats induced by these nanoparticles were investigated after intratracheal instillation in male Wistar rats. Moreover, the relationships between the particle size, particle crystalline structure, chemical composition, chemical stability and toxicological effects of these typical nanoparticles in rats were explored. Exposure to nanoparticles increased the oxidative stress level in peripheral blood and the homogenates of the liver, spleen and thymus as well as disorders in regulating the cytokine network and blood cell count in the peripheral blood. Furthermore, the histopathological study revealed that pulmonary exposure to nanoparticles produced persistent, progressive liver inflammatory responses and cell necrosis, while no observable damage was found in the kidney, thymus gland or spleen tissue from the experimental groups. Our results demonstrate that oxidative stress might be important for inducing the toxic effects of these nanoparticles, and three nanoparticles can influence the

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immune function of rats. A comparative analysis of the toxic effects of nanomaterials demonstrated significant differences. Nano-ZnO induced the most significant toxicity, whereas Nano-TiO_2 induced the least. Particle composition and chemical stability probably played a primary role in the toxicological effects of different nanoparticles.

Introduction

In recent years, nanotechnology and nanoscience has progressed by leaps and bounds, and various types of manufacture materials and nanoparticles are being rapidly produced and widely applied in large quantities worldwide. With the increased probability of exposure from large-yield industry production and the growing number of applications for nanomaterials, the hazards of nanoparticles have been a focus of research. Because of their unique nano-scale, nanoparticles have many special physicochemical properties, such as a small size, large surface area and high reactivity; therefore, compared to normal particles, they may have extraordinary biotoxicity for human health [1-3]. Meanwhile, owing to their size and unusual properties, nanoparticles can enter the body and cross biological barriers relatively unimpeded. Several studies have reported that nanoparticles enter the body through the respiratory tract and can penetrate the pulmonary epithelium; they can reach the interstitium and be deposited in the peripheral lung tissue [4,5]. They can cross the pulmonary blood barrier and gain access to the blood circulation. Once they are in the circulatory system, they can be transferred to the liver and other tissues/organs, where they could accumulate and damage organ systems [6].

Silver, zinc oxide, and titanium dioxide nanoparticles are widely used as sterilization materials to enhance the performance of disinfectants [7]. Although there are a wide and growing number of applications for these three types of nanomaterials, there is a serious lack of information on the comparative study of toxicity in human

health and the environmental implications of these manufactured nanomaterials. Limited studies assessing the inter-relationship between the toxicity and properties of manufactured nanomaterials are available. The objective of the present study was to explore toxicity in rats induced by silver, zinc oxide, and titanium dioxide nanomaterials (Nano-Ag, Nano-ZnO and Nano-TiO_2).

Oxidative stress is a well-defined paradigm to explain the toxic effects induced by nanomaterials [8]. The present study also focused on the oxidative effect induced by nanoparticles. Therefore, the levels of malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH) and nitrogen oxide (NO) in the peripheral blood, liver (metabolic organ), spleen (peripheral immune organ) and thymus gland (central immune organ) homogenates were measured. Simultaneously, histopathological examinations of the above organs were also performed. In addition, the balance of the cytokine network plays an important role in maintaining the immune and inflammatory responses of organisms. Hence, the levels of interleukin (IL)-1, tumor necrosis factor-alpha (TNF-α) and interferon (IFN)-γ in the serum of rats exposed to nanomaterials were measured to illustrate the effects of nanoparticles on the rat. This study provides informative results on the toxicity of the three types of studied nanoparticles, which may extend our understanding of the toxicity of disinfectants.

Materials and methods

Nanoparticles and suspension preparation

Manufactured nanoparticles of TiO_2 and Ag were purchased from Sigma–Aldrich. Nano-ZnO was purchased from Shenzhen Nanuo Nanomaterials Crop. The particles were prepared in fetal bovine serum (FBS; Gibco, Billings, MT, USA) by vortexing

the suspension 10 times for 10 seconds, which was followed by sonication (ten times, 30 s every 2 min) at 4 ℃ to break down agglomerates and ensure a uniform suspension. The particle size and shape were measured by transmission electron microscopy (TEM) (JEM-100CX, Japan) (Fig. 1). The particle specific surface area (SSA) was determined with a BET surface area analyzer (AUTOSORB-MP). The crystal structure of the nanoparticles was characterized using X-ray diffraction (XRD) (RIGAKU, Mini Flex Ⅱ) (Fig. 2). In this experiment, the titanium dioxide nanoparticles were an anatase-rutile mixture; however, anatase nanoparticles constitute the majority of them. The zeta potential is measured by applying an electrical field through the solution, and, depending on the zeta potential, particles with different charges will migrate at different speeds toward the electrodes, which is measured and converted to the zeta potential using Smoluchowski's theory (Beckman Coulter DelsaTMNano C). The zeta potential was measured in MilliQ water with 1 mM NaCl as described in Hanna et al [9]. The density and composition are from the suppliers' datasheets. The properties of the nanoparticles are summarized in Table 1.

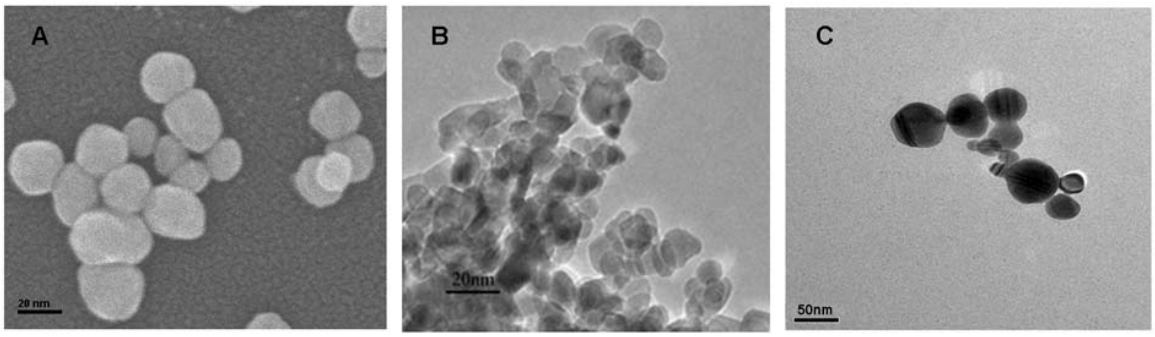

Fig. 1 Images of typical particles of (A) Nano-TiO_2; (B) Nano-ZnO; and (C) Nano-Ag by TEM

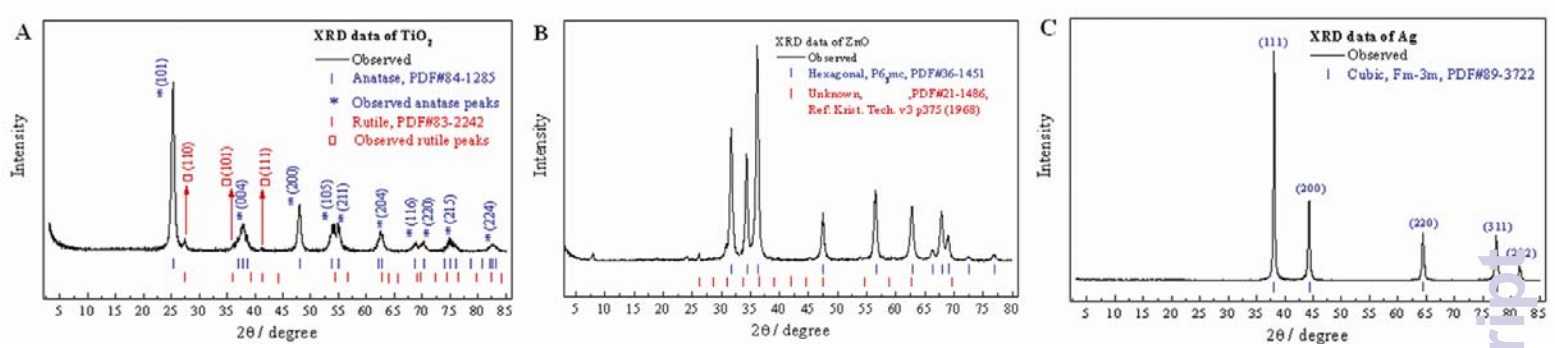

Fig.2 XRD of (A) Nano-TiO_2; (B) Nano-ZnO; and (C) Nano-Ag

Table 1 Characterization of the three nanomaterials

Particle	Zeta potential (mV)	Size (nm)	Density (g/cm^3)	SSA (m^2/g)	Crystalline structure	Shape	Composition
Nano-Ag	4.9	52.25±23.64	10.49	5	Cubic	Sphere	Ag >99.5%
Nano-ZnO	18.63	19.61±5.83	5.78	45	Hexagonal	sphere	ZnO >99.9%
Nano-TiO_2	-25.8	22.82±5.30	4.26	35-65	tetragonal	Sphere	TiO_2 >99.7%

Animals and treatment

Forty-two healthy male Wistar rats (4 weeks of age, weighing 160–180 g) were obtained from the Academy of Military Medical Sciences (Beijing, China). The study protocol was approved by the Chinese Association for Laboratory Animal Science. The rats were randomly divided into the following seven groups: control group and 3.5 mg/kg BW (Nano-ZnO^L, Nano-Ag^L and Nano-TiO_2^L) or 17.5 mg/kg BW (Nano-ZnO^H, Nano-Ag^H and Nano-TiO_2^H) dosage groups for the three nanomaterials. One week prior to the beginning of the experiment, the rats were housed in pairs under controlled environmental conditions (temperature 24 ± 1°C, humidity 50 ± 5%, lights on 07:00-19:00 h). The treatment was performed in a Grade

II animal room, and there were no other air pollutants in the environment. Each group received an intratracheal instillation once every 2 days for 5 weeks.

Oxidative stress and immune inflammatory level assay in peripheral blood

Blood samples were collected from the orbit venous plexus approximately 24 h after the last treatment. The cell classification count in the blood samples was detected with an automatic blood biochemical analyzer (TBA-120 FR, Toshiba). In addition, the serum was prepared immediately and stored at 20 ℃. The levels of GSH, SOD, MDA and NO in serum were detected using reagent kits purchased from Jiancheng Bioengineering (Nanjing, China) according to the manufacturer's instructions. In addition, IL-1, INF-γ and TNF-α in serum were measured using a double-antibody sandwich enzyme linked immunosorbent assay (ELISA) kit that is specific for rats (R&D Systems, Minneapolis, MN, USA). The assays were performed according to the manufacturer's instructions. The optical density was measured using an Automatic Multi-function Microplate Reader (Multiskan MK3; Thermo Scientific, Waltham, MA, USA) at 450 nm.

Collection and analysis of the liver, spleen and thymus gland homogenates

Portions of the livers, spleens and thymus gland of each rat were excised, washed in ice-cold physiological saline, weighed, and transferred into a centrifuge tube; then, 1:9 (w/v) volume of cold phosphate-buffered saline (PBS) was added, and the sample was homogenized using a ultrasonic cell disruptor (Sonics vibra cell, VCX105) for 8 s×4 times at 4 ℃. The homogenates were then centrifuged at 2500 rpm at 4 ℃ for 15 min. The supernatants were collected to analyze the GSH, SOD and MDA levels.

Histopathological examination of the liver, kidney, spleen and thymus gland

Portions of the liver, kidney, thymus gland and spleen of the rats exposed to three types of nanoparticles were collected and immediately fixed in 10% buffered formalin for at least 5 days. Each tissue was processed in an automatic issue processor and embedded in paraffin. Thin sections were cut at a thickness of 5 μm and then mounted onto glass slides. After staining with hematoxylin-eosin, the pathological evaluation was performed using a microscope.

Statistical analyses

Test data for statistical treatment were evaluated using SPSS software (version, 13.0). The data were expressed as the mean ± SEM (standard error of mean). The results were evaluated using ANOVA, which was followed by the least post squares (*post hoc* test*)* (equal variances) or Dunnet's T3 *post-hoc* test (unequal variances). A significant difference was considered for $p<0.05$.

Results

Oxidative-stress-related biomarker in the peripheral blood

The balance of oxidants and antioxidants is essential to healthy organisms. The concentrations of MDA and NO were monitored to elucidate the lipid peroxidation induced by nanomaterials. As shown in Figure 3, three types of nanoparticles can significantly increase the concentrations of MDA and NO in serum. In addition, the GSH and SOD levels were measured to evaluate the antioxidative response of the rats to nanoparticles. Figure 2 shows that the GSH concentrations of all exposure groups were significantly lower than that of the control group. The SOD activities in the

Nano-ZnO^{H}, Nano-ZnO^{L} and Nano-Ag^{H} exposure groups were significantly lower than that of control group. There was a dose–dependent relationship between the concentration of Nano-ZnO and the levels of MDA and GSH. Moreover, comparative analyses of these oxidative effects illustrated significant differences among the three types of nanomaterials. Nano-ZnO induced the most significant oxidative stress, whereas Nano-TiO_2 induced the least.

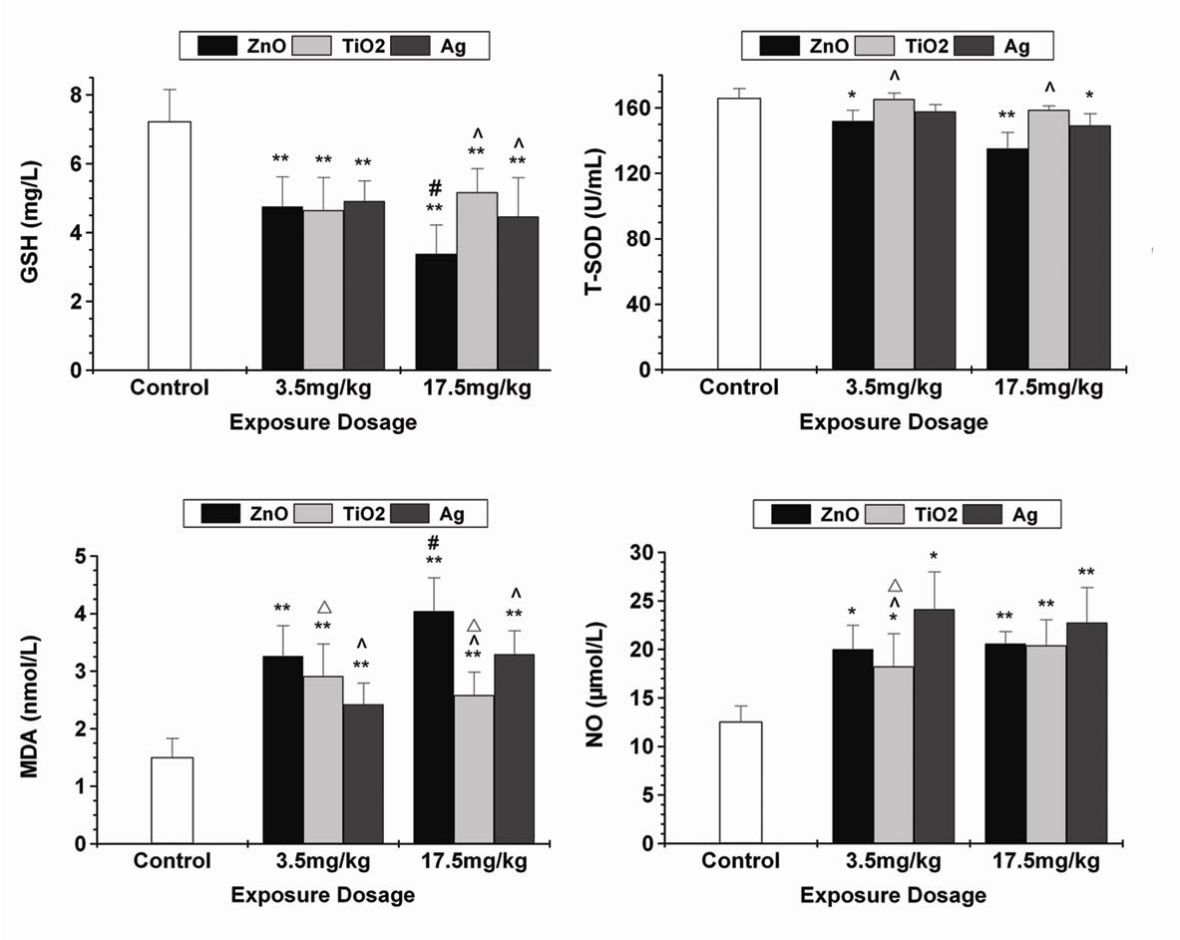

Fig. 3 Oxidative stress levels in the serum of rats exposed to three types of nanoparticles. $^{*}p$ <0.05, $^{**}p$ <0.01 versus control; $^{\#}p$ <0.05 versus low-dose for the same nanoparticles; $^{\wedge}p$ <0.05 versus Nano-ZnO at the same dose; and $^{\triangle}p$ <0.05 versus Nano-Ag at the same dose.

Cytokine levels in the peripheral blood

As shown in Figure 4, except for the low-dose Nano-TiO_2 and low-dose Nano-ZnO

subgroups, the concentrations of IL-1 in serum increased significantly in the nanoparticle exposure groups. The IFN-γ concentrations in the high-dose Nano-TiO_2 and high-dose Nano-Ag subgroups decreased significantly. The TNF-α concentrations in the Nano-ZnO^L, Nano-$TiO_2{}^H$ and Nano-$TiO_2{}^L$ exposure groups decreased significantly; however, the level of TNF-α increased significantly in low-dose Nano-Ag subgroup.

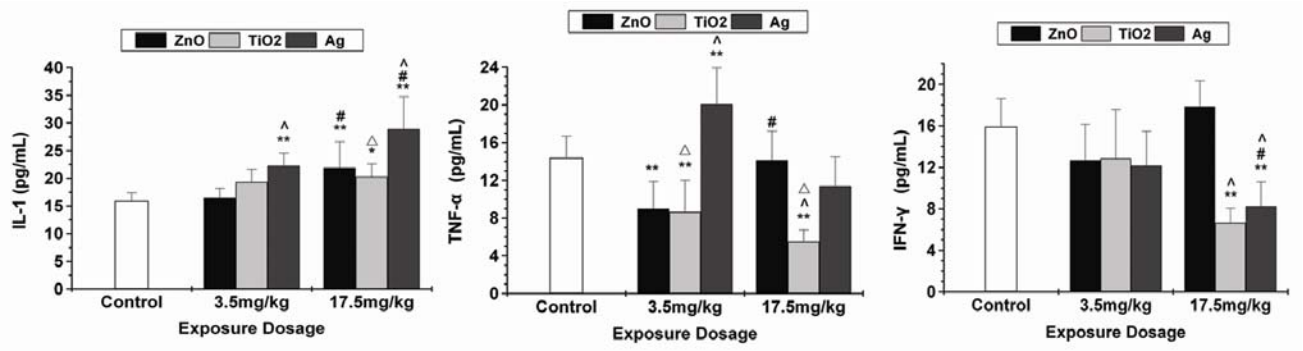

Fig. 4 Cytokine levels in the serum of rats exposed to three types of nanoparticles. $^{*}p$ <0.05, $^{**}p$ <0.01 versus control; $^{\#}p$ <0.05 versus low-dose in the same nanoparticles; $^{\wedge}p$ <0.05 versus Nano-ZnO at the same dose; and $^{\triangle}p$ <0.05 versus Nano-Ag at the same dose.

Cell count and classification in peripheral blood

Table 2 shows that the red blood cell (RBC) counts in the peripheral blood of the high-dose Nano-Ag subgroup was significantly higher than that of the control group. The white blood cell (WBC) counts in the high-dose Nano-ZnO and low-dose Nano-Ag subgroups increased significantly; however, the WBC counts decreased significantly in the other exposure groups. The percentage of lymphocytes (LYM) in the high-dose Nano-Ag and low-dose Nano-ZnO subgroups were significantly decreased. The percentage of monocytes (MON) in the high-dose Nano-ZnO subgroup and the percentage of neutrophils (NEUT) in low-dose Nano-ZnO and

Nano-TiO_2 subgroups were significantly increased.

Table 2 Classification count of white blood cells in the peripheral blood of rats exposed to three types of nanoparticles

Group	RBC (×10^9/mL)	WBC (×10^6/mL)	LYM (%)	MON (%)	NEUT (%)
Control	7.72±0.48	13.0±1.5	62.2±4.0	15.8±0.9	22.0±4.5
Nano-ZnO^H	8.11±0.67	14.2±2.5	53.4±9.5	21.6±8.8	25.2±8.6
Nano-TiO_2^H	7.97±0.54	8.4±0.7**	60.2±10.9	13.1±3.1	26.7±10.4
Nano-Ag^H	8.46±0.44*	8.8±1.4**	53.0±6.4*	15.6±2.8	31.4±4.6
Nano-ZnO^L	7.92±0.49	8.8±1.9**	52.2±9.5*	9.9±2.2**	37.8±7.9*
Nano-TiO_2^L	7.65±0.75	8.7±1.5**	54.3±3.6	13.3±2.4	32.4±2.5*
Nano-Ag^L	8.19±0.56	10.3±1.4**	61.2±4.5	16.2±2.4	22.6±3.5

Oxidative-Stress-related biomarker in the liver, spleen and thymus gland homogenates

The oxidative stress level in the liver homogenates of rats exposed to nanoparticles is shown in Figure 5. The SOD activity and GSH content in all exposure groups decreased significantly compared with the control group. The MDA concentration in all nanoparticle groups increased significantly.

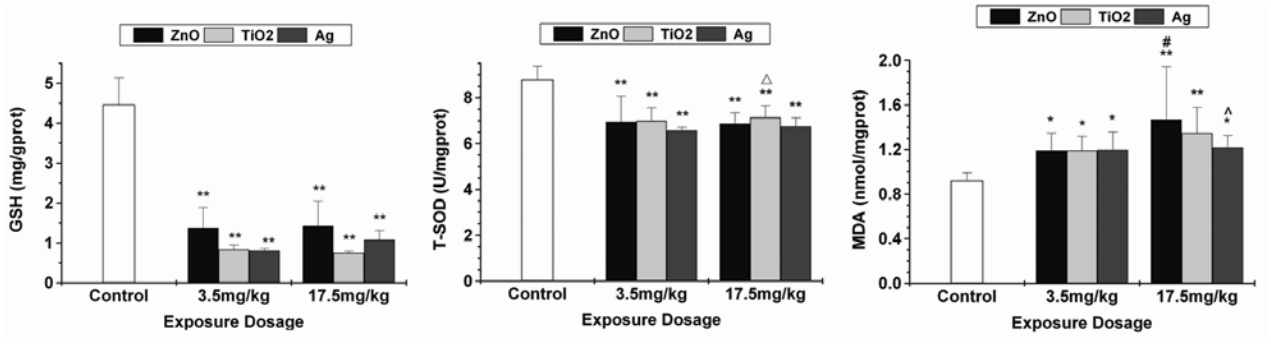

Fig. 5 Levels of oxidative stress in the liver of rats exposed to three types of nanoparticles. *p

<0.05, $^{**}p$ <0.01 versus control; $^{\#}p$ <0.05 versus low-dose in the same nanoparticles; $^{\wedge}p$ <0.05 versus Nano-ZnO at the same dose; and $^{\triangle}p$ <0.05 versus Nano-Ag at the same dose.

Figure 6 shows the oxidative stress level in the spleen homogenates of rats exposed to nanoparticles. The GSH concentration in the Nano-ZnO exposure groups and SOD activity in the two-dose Nano-ZnO and high-dose Nano-Ag subgroups decreased significantly. The MDA concentration in all exposure groups increased significantly. Furthermore, the oxidative stress levels in the spleen of rats induced by Nano-ZnO and Nano-Ag were greater than that for Nano-TiO_2.

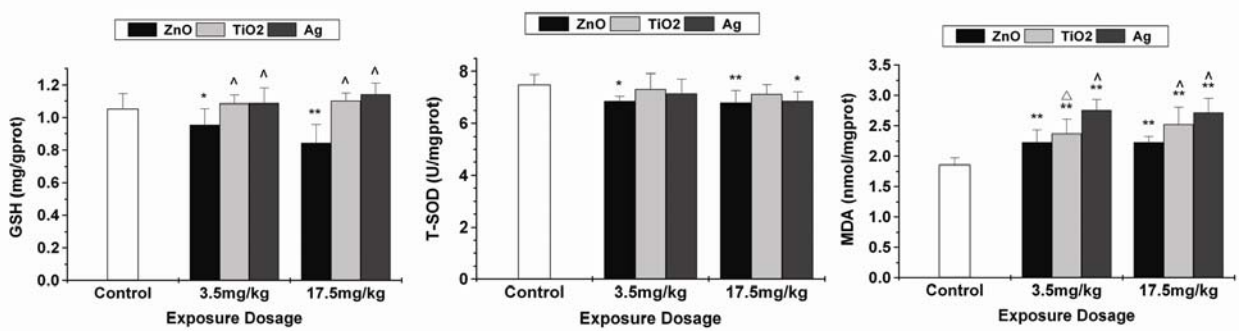

Fig. 6 Levels of oxidative stress in the spleen of rats exposed to three types of nanoparticles.

$^{*}p$ <0.05, $^{**}p$ <0.01 versus control; $^{\wedge}p$ <0.05 versus Nano-ZnO at the same dose; and $^{\triangle}p$ <0.05 versus Nano-Ag at the same dose.

Figure 7 shows the oxidative stress level in the thymus gland homogenates of rats exposed to nanoparticles. The SOD activity in all exposure groups and the GSH concentration in the high-dose Nano-ZnO and high-dose Nano-Ag subgroups decreased significantly. The MDA concentration in the two-dose Nano-Ag and high-dose Nano-ZnO subgroups increased significantly. A dose-dependent relationship between the SOD activities and concentrations of Nano-ZnO and Nano-TiO_2 was observed. As observed with oxidative stress factors in the spleen, the

oxidative stress in the thymus gland homogenates of rats induced by Nano-Ag and Nano-ZnO was greater than from Nano-TiO_2.

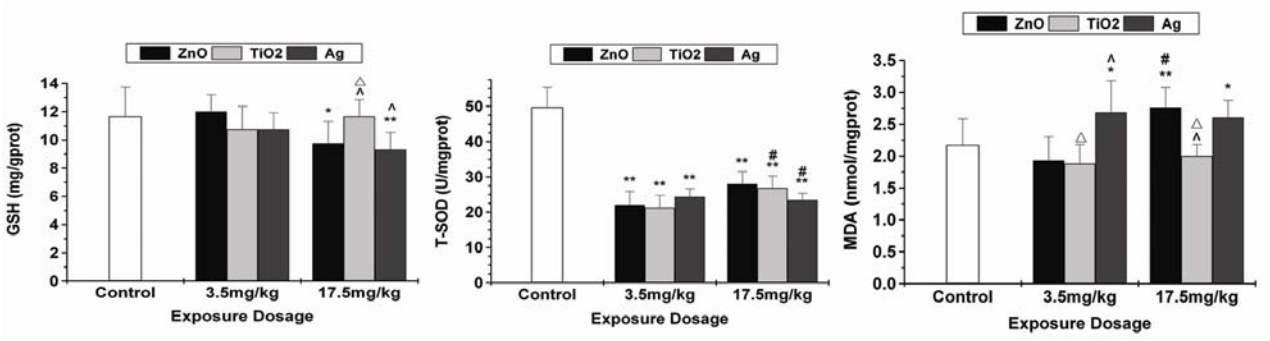

Fig. 7 Levels of oxidative stress in the thymus gland of rats exposed to three types of nanoparticles. $^{*}p$ <0.05, $^{**}p$ <0.01 versus control; $^{\#}p$ <0.05 versus low-dose in the same nanoparticles; $^{\wedge}p$ <0.05 versus Nano-ZnO at the same dose; and $^{\triangle}p$ <0.05 versus Nano-Ag at the same dose.

Histopathological findings

There were no nanoparticle-related gross lesions and microscopic changes observed in any of the observed organs of the treated rats, except for the liver. The histological changes of the livers in the control group after treatment revealed no observable damage (Figure 8A). In contrast, pulmonary exposure to nanoparticles produced persistent and progressive liver inflammatory responses. The livers of the exposure groups (Figure 8B, C, D) produced obvious inflammatory cell infiltration, liver cell fatty degeneration, and focal liver cell degeneration and necrosis in the liver centrilobular portion, especially in the Nano-ZnO and Nano-Ag groups (Figure 8B, D). The histopathological lesions induced by Nano-ZnO involve the overall liver tissue. However, these effects were absent in the kidney, thymus gland and spleen tissue from the experimental groups.

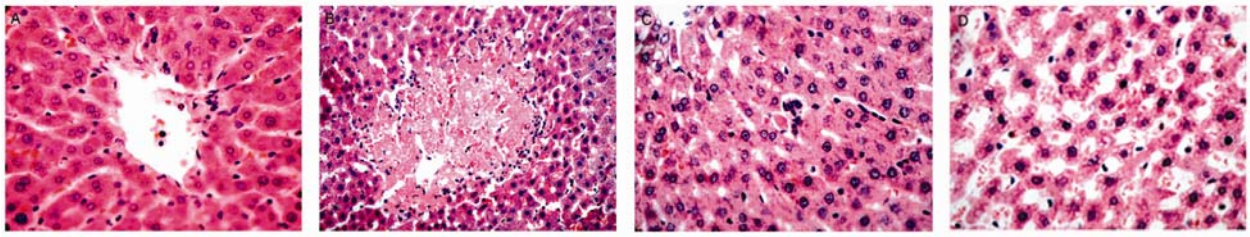

Fig. 8 Light micrographs of liver tissue of rats exposed to three types of nanoparticles. A. Control group liver; B. Nano-ZnO^{H} group liver; C. Nano-$TiO_2{}^{H}$ group liver; and D Nano-Ag^{H} group liver. Magnification, ×40.

Discussion

The toxicity and underlying mechanism of action

Nanomaterials are small with large surface areas; therefore, they have a stronger ability to penetrate tissue and cells as well as have higher oxidative and catalytic ability than normal particles [10]. Oxidative stress and free radicals can induce damage to lipids and cell membranes, causing cell apoptosis or death [11]. MDA is the most representative product of lipid peroxidation within cells, and the MDA concentrations can indicate the rate and intensity of lipid peroxidation within the body [12]. GSH concentrations and SOD activity directly reflect the antioxidant levels of the cell and body. As a new type of immune molecule and inflammatory mediator, NO can mediate the cytotoxic effects and immune regulation *in vivo*. It can mediate the pathological role of toxins, IL-1β, IL-6, TNF-α and other cytokines. Moreover, NO can also react with superoxide anions to generate peroxide and nitroso anions that cause severe oxidative damage to the body [13].

The liver tissue is responsible for detoxication and when the organisms are exposed to foreign substances, the liver is one of the first tissues to suffer damage.

The immune system is sensitive to external stimuli. The adverse effects on the immune system induced by test substance are mainly reflected in the morphology and functional changes of the immune organs and immune cells. The thymus gland and spleen are the main immune organs of an organism. The thymus, a primary lymphoid organ, influences and regulates the differentiation, development and function of T lymphocytes by secreting thymic hormone. It has an important role in regulating the immune balance of the organism and maintaining the autoimmune stability. The spleen, a secondary lymphoid organ, is the body's largest lymphoid organ, which contains lymphocytes and macrophages. It is closely related to both humoral and cellular immunity, and it plays an anti-tumor role through a variety of mechanisms. In the present study, all nanoparticles caused a depletion of the GSH content, reduction in the SOD activity, and increase in the concentrations of MDA to the liver. This phenomenon was also observed in the immune organs, the spleen and thymus gland, of the rats exposed to nanoparticles. Therefore, the results suggested that tissues and cells were attacked by free radicals so that the antioxidant levels and ability to scavenge free radicals were significantly decreased. Simultaneously, exposure to three types of nanoparticles also caused a depletion of GSH content, reduction in SOD activity (except for the Nano-TiO_2 and low-dose Nano-Ag subgroups) and increase in the concentrations of MDA and NO in peripheral blood, further substantiating that oxidative stress might have a crucial role in inducing the toxicity of nanoparticles.

The count and classification count of white blood cells in the peripheral blood can reflect the effects of test substances on the immune cells in the blood,

inflammatory reaction condition of organisms and hematopoietic system. White blood cells, known as immune cells, can swallowed foreign materials and produce antibodies, heal damage to the organism, resist the invasion of pathogen and adjust the immune function of organism. WBCs can be divided into five categories, including lymphocytes, monocytes, neutrophils, eosinophils (EOs) and basophils (EASOs). Neutrophils and monocytes have very strong phagocytosis, and they mainly play a role in nonspecific immunity. Lymphocytes are responsible for humoral immunity, cellular immunity and the secretion of lymphokines. They play a major role in the organism-specific immune process. The inflammation or other diseases can cause changes in the WBC totality and percentage of various WBCs. The present study indicates that three types of nanoparticles can induce the immune response and inflammation reaction and have adverse effects on the immune function of the organism. These effects exhibited specificity in different materials, different exposure doses and different times. Combined with the results of oxidative damage on the spleen and thymus in the current study, we suggest that these nanoparticles induced toxic damage on immune organs, potentially leading to immune cell death or production/maturation disorder.

The balance of the cytokine network plays an important role in maintaining the immune and inflammatory responses of organisms. It can regulate the enhancement or inhibition of immune responses. The content of cell factors and inflammatory cytokines in peripheral blood serum is a reflection of overall immune system function and the level of inflammatory reaction. The present study showed that all three types

of nanoparticles could induce the generation of immune regulation responses. The increase in the nonspecific immune function of rats was enhanced by stimulating the up-regulation of IL-1. The low-dose Nano-Ag could boost the function of nonspecific immune system by up-regulating IL-1 and TNF-α. However, high-dose of Nano-Ag and Nano-TiO_2 could decrease the nonspecific immune function of organisms by depressing the release of IFN-γ and TNF-α. These results suggested that if an organism is exposed to nanoparticles, with different sizes, chemical compositions and doses, the cytokines and inflammatory mediators for the response related to the immune regulation mechanism of the organism are different. Numerous studies have demonstrated that upon stimulation with a foreign material, the body may first respond with immune regulation, showing immunostimulation. The immune regulation network is destroyed as long as the extension of the exposure time and exposure dose, which is followed with the immune suppression [14]. Research has shown that the influence of metal/metal-oxide on the function of cellular immunity is bidirectional, which is an immune stimulating effect when it comes with low dose, while it shows obvious inhibition at high dose [14]. The detailed mechanisms require further experimental studies.

Three types of nanoparticles were found to affect the histopathology of the rat liver, suggesting that these nanoparticles have hepatotoxic abilities in rats. Nevertheless, no changes were observed in the pathology of the kidney, thymus gland and spleen. We speculate that the reaction in oxidative stress, including biochemical markers of early tissue damage, did not affect the cellular integrity or tissue

morphology. Wu et al. [15] also found no abnormal pathology changes in the striatum and hippocampus 7 days after instillation with SiO_2 nanoparticles, although the reaction in oxidative stress and increase in cytokines were observed in these tissues.

In our previous study, exposure to the three types of nanoparticles by intratracheal instillation induced severe oxidative stress, an inflammation response, and histopathology changes in the lung tissues of rats [13]. Combined with the results of the current study, these nanoparticles deposited in the lung tissue induced inflammation in the lung, and this process leads to the release of high levels of inflammatory cytokines and chemokines into the circulatory system, resulting in systemic oxidative injury and an inflammatory response involving the liver, spleen, and thymus gland. Meanwhile, nanoparticles can cross the pulmonary-blood barrier and gain access to the blood circulation; once in the circulatory system, they can be transported into the liver and other tissues/organs to induce toxic effect.

Relationship between the characteristics of nanoparticles and their toxic effects

A comparative analysis of the toxic effects of nanomaterials demonstrated significant differences. Nano-ZnO induced the most significant toxicity, whereas Nano-TiO_2 induced the lowest toxicity. The toxicity of nanoparticles may be determined by several factors. In general, it is believed that the smaller the particle size for a given material, the greater the toxic effect [2,16-18]. It has been reported that the toxicological effects of different nanoparticles can be attributed to their surface properties, which originate from the specific “nano” size effect, but they are ultimately determined by the chemical composition [19-21]. However, the chemical

composition of nanomaterials is most likely the dominant factor in their toxic effects [22]. Moreover, metal or metal-oxide classes of nanoparticles can respond and dissolve rapidly as soon as they ingress into cells. The intermediate products also likely cause the generation of free radicals, which directly access certain organelles to induce ultrastructural changes and damage cell function [23].

In these experiments, Nano-Ag was a metal nanomaterial and Nano-ZnO and Nano-TiO_2 were metal-oxide nanomaterials. The toxicity of metal or metal-oxide nanomaterials is related to their chemical stability. Nanomaterials with good chemical stability have no significant toxicity, whereas readily soluble nanometals with redox potential have significant cytotoxic (or even genotoxic) properties [24]. Nano-ZnO is slightly soluble; after entering the organism, it can be ionized to release Zn^{2+}, which may access cells through ion channels. Subsequently, Zn^{2+} can enter the blood circulation to damage the cell metabolism and stimulate cells to produce high levels of ROS, causing oxidative stress and reducing mitochondrial function, LDH leakage, and DNA damage and inducing apoptosis [25, 26]. Because of its large, specific surface area, when Nano-Ag exposed to water or in water environment, its surface can be hydrolyzed and release silver ions [27]. The silver ions that enter cells can induce the generation of superoxide free radicals and other reactive oxygen free radicals, which may cause oxidative stress and cell membrane damage to cells. Silver nanoparticles that enter cells can lead to cell apoptosis or necrosis, resulting in cell death [28]. Kim et al. reported that the silver nanoparticles in liquid culture can release tiny amounts of silver ions, but their findings suggest that Nano-Ag cytotoxicity is primarily due to oxidative stress and is independent of the toxicity of Ag(+) ions [27]. Other studies that compared the toxicity of carbonate silver or silver nitrate and silver nanoparticles confirmed that ionic Ag+ measured in the Nano-Ag suspensions could not fully

explain the toxicity of Nano-Ag; the interaction of Nano-Ag particles with cells influences on the toxicity of Nano-Ag, which is mediated by Ag+; both "nanosized particle of Ag" as well as "ionic Ag+" contribute to the toxic effects of Nano-Ag [29-31]. Nano titanium dioxide cannot release the soluble ions in aqueous solution [32]. The difference in the lighting conditions and crystal structure are the main factors that influence its biological effect in addition to the particle size. When under ultraviolet illumination, the surface of the titanium dioxide nanomaterial can generate reactive oxygen species with strong oxidation ability, causing cell damage. Previous studies found that anatase Nano-TiO_2 can more effectively promote the generation of reactive oxygen species, inflammatory response and cytotoxic injury compared to the rutile Nano-TiO_2 [33, 34]. However, Gurr et al. found that rutile-sized 200-nm titanium dioxide particles could induce hydrogen peroxide and oxidative DNA damage in the absence of light, while the anatase-sized, 200-nm particles could not cause such effects [35]. The precise toxicity mechanism of titanium dioxide nanomaterials should be studied in the future.

In the present study, Nano-ZnO and Nano-TiO_2 had similar particle sizes and shape; therefore, the differences in toxicity could likely be attributed to their chemical compositions, chemical stability and crystalline structure. Despite having the smallest particle size and largest SSA, Nano-TiO_2 exhibited much lower toxic effects than Nano-Ag. Therefore, it seems that the toxicological effects of different nanoparticles may be attributed to their surface properties, which originate from the specific nano size and chemical compositions, but they are ultimately determined by particle size.

5. Conclusion

Notwithstanding its limitations, the present study demonstrated that oxidative

stress and the inflammation response might be important for inducing the toxic effects of nanoparticles, and the toxic effects of various nanoparticles were different. Our results suggested that the particle surface properties, determined by chemical composition, could have a primary role in the toxicological effects of different nanomaterials. However, the precise transduction pathways and mechanisms of oxidative stress induced by nanoparticles are not well defined. Future investigations may improve our understanding of the relationship between the surface properties and cellular uptake, reaction pathways and oxidative stress mechanisms of nanoparticles *in vivo* and *in vitro*.

Acknowledgements

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