

Lung Tissue Alterations were Size-dependent with Smaller Ones Induced More Effects and Related with Time Exposure of Gold Nanoparticles

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Abstract

Background: The aim of the present study was to investigate the effects of intraperitoneal administration of GNPs on the histological alterations of the lung tissue of rats in an attempt to cover and understand the toxicity and the potential role of GNPs as a therapeutic and diagnostic tool.

Methods: A total of 40 healthy male Wistar-Kyoto rats were exposed to GNPs received 100 μ l of GNPs infusion of 10, 20 and 50 nm GNPs for 3 or 7 days. Animals were randomly divided into groups, 6 GNPs-treated rat groups and one control group (CG). Groups 1, 2, 3 received infusion of 100 μ l GNPs of size 10 nm (3 or 7 days), size 20 nm (3 or 7 days) and 50 nm (3 or 7 days), respectively.

Results: GNPs-treated rats receiving 100 μ l of 10 and 20 nm particles for 3 days demonstrated interstitial chronic inflammatory cell infiltration with extravasation of red blood cells and thickened alveolar wall and congested blood vessels while 50 nm GNPs-treated rat demonstrated interstitial inflammatory cells infiltrate with small lymphocytes, plasma cells and considerable number of eosinophils and surrounded by congested and dilated blood vessels. GNPs-treated rat which received 100 μ l of 10 and 20 nm particles for 7 days demonstrated diffuse interstitial lung pneumonia with small lymphocytes and plasma cells, considerable number of eosinophils, scattered extravasation of red blood cells surrounded by thickened, dilated and congested blood vessels while 50 nm GNPs-treated rat demonstrated dense interstitial inflammatory cells infiltrate of chronic type.

Conclusions: The alterations induced by intraperitoneal administration of GNPs were size-dependent with smaller ones induced more affects and related with time exposure of GNPs. This study suggests that GNPs may interact with proteins and enzymes of the lung tissue interfering with the antioxidant defense mechanism and leading to reactive oxygen species (ROS) generation.

Keywords: Gold nanoparticles; Size; Lung tissue; Histology; Inflammatory; Nanotoxicity; Rats

Introduction

The NPs may differ in reactivity and solubility and may interact with all kinds of endogenous proteins, lipids, polysaccharides and cells. GNPs can easily enter cells and the demonstration that amine and thiol groups bind strongly to GNPs has enabled their surface modification with amino acids and proteins for biomedical applications [1,2].

GNPs are thought also to be relatively non-cytotoxic [3] while the metallic nature of the metal derived NPs and the presence of transition metals encourages the production of reactive oxygen species (ROS) leading to oxidative stress [4,5]. There are differing reports of the extent of the toxic nature of these particles owing to the different modifications of the GNPs, surface functional attachments and shape and diameter size of the NPs [6,7].

The particle size-dependent organ distribution of GNPs has been studied *in vivo* [8,9]. *In vivo* studies in rats exposed to aerosols of GNPs revealed that the NPs were rapidly taken into the system with the highest accumulation in the lungs, aorta, esophagus and olfactory bulb [10].

In order to understand and categorize the mechanisms for GNPs toxicity, information is needed on the response of living systems to the presence of GNPs of varying size, shape, surface, bulk chemical composition, and exposure duration. Very little information on these aspects is presently available and this implies that there is an urgent need for histological data for GNPs.

The histological and histochemical characterization in the lung

tissues due to GNPs has not been documented and identified. In the present study, an attempt has been made to characterize the possible histological alterations in the lung tissues after intraperitoneal administration of GNPs and, if so, whether are related to the size of these GNPs and the time of exposure.

Material and Methods

A total of 40 healthy male Wistar-Kyoto rats were obtained from the Laboratory Animal Center (College of Pharmacy, King Saud University, Saudi Arabia). The rats nearly of the same age (12 weeks old) and weighing 220-240 g of King Saud University colony were used. Animals were randomly divided into groups, 3 GNPs-treated rats groups and one control group (CG). The 10, 20 and 50 nm GNPs were administered intraperitoneally at the rate of 3 or 7 days as follows: Group 1: received infusion of 100 μ l GNPs of size 10 nm for 3 or 7 days (n = 10); Group 2: received infusion of 100 μ l GNPs of size 20 nm for 3

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or 7 days (n = 10); Group 3: received infusion of 100 µl GNPs of size 50 nm for 3 or 7 days (n =10). Control group: received no GNPs (n =10).

The rats were maintained on standard laboratory rodent diet pellets and housed in humidity and temperature-controlled ventilated cages on a 12 h day/night cycle. All experiments were conducted in accordance with the guidelines approved by King Saud University Local Animal Care and Use Committee.

Fresh portions of the lung from each rat were cut rapidly, fixed in neutral buffered formalin (10%), then dehydrated, with grades of ethanol (70, 80, 90, 95 and 100%). Dehydration was then followed by clearing the samples in 2 changes of xylene. Samples were then impregnated with 2 changes of molten paraffin wax, then embedded and blocked out. Paraffin sections (4-5 µm) were stained with hematoxylin and eosin (the conventional histological stain) according to Pearse [11]. The bright-field images were acquired using a Nikon Eclipse 800 microscope equipped with a Nikon DXM1200 color CCD camera (Nikon Instruments Inc., Melville, NY).

Stained sections of control and treated rats were examined for alterations in the lung tissues for the presence of lung pneumonia with small lymphocytes, plasma cells and eosinophils, extravasation of red blood cells, alveolar spaces, and congestion and dilation of blood vessels.

Results and Discussion

Size and morphology of different gold nanoparticles (GNPs)

The 10 and 20 nm GNPs show spherical shape while the 50 nm GNPs show hexagonal shape. The mean size for GNPs was calculated from the images taken by the transmission electron microscope (TEM): The 10 nm GNPs was of mean size 9.45 ± 1.33 nm, 20 nm GNPs was of mean size 20.18 ± 1.80 and the 50 nm GNPs was of mean size 50.73 ± 3.58 [12-15].

Histological alterations

No mortality occurred for the administration periods 3 and 7 days of GNPs in any of the experimental groups of the present investigation, and no alterations were observed in the appearance and behavior of GNPs treated rats in comparison with the control ones.

Control group (Figure 1): Microscopic pictures show GNPs-normal rat demonstrated well-formed and opened alveoli with its normal appearing septum, few scattered small lymphocytes and minimal eosinophils and with no other pathological changes.

In comparison with the control group, the following histological alterations were detected in the lung tissue of GNPs treated rats. These histological alterations were observed in Figures 2-7. The histological alterations can be summarized as follows:

1) GNPs-treated rat receiving 100 µl of 10 nm particles for 3 days demonstrated interstitial chronic inflammatory cells infiltrate, considerable amount of scattered red blood cells with patent alveolar spaces, and thickened congested blood vessels as shown in Figure 2.

2) GNPs-treated rat receiving 100 µl of 10 nm particles for 7 days demonstrated diffuse interstitial lung pneumonia with small lymphocytes and plasma cells, considerable number of eosinophils, scattered extravasation of red blood cells surrounded by thickened blood vessels, and patent alveolar spaces as shown in Figure 3.

3) GNPs-treated rat receiving 100 µl of 20 nm particles for 3 days

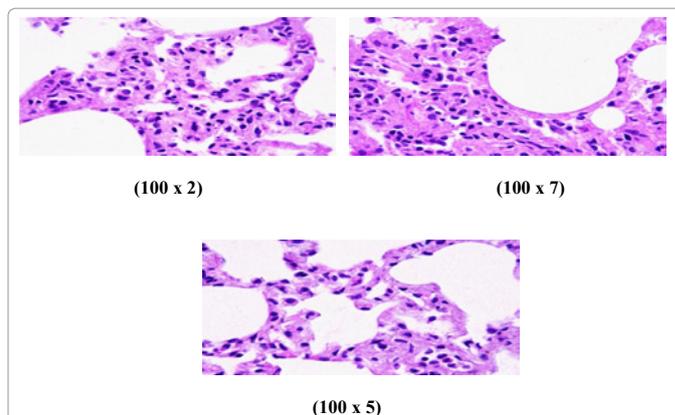


Figure 1: GNPs-normal rat demonstrated well-formed and opened alveoli with its normal appearing septum, few scattered small lymphocytes and minimal eosinophils, and with no other pathological changes.

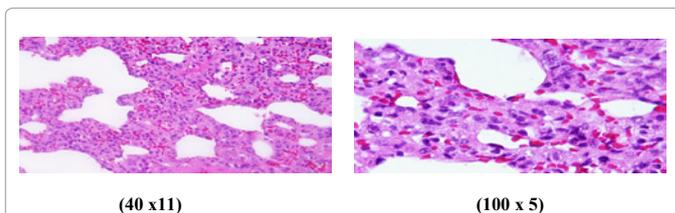


Figure 2: GNPs-treated rat received 100 µl of 10 nm particles for 3 days demonstrated inflammatory cells infiltrate with extravasation of red blood cells and with thickened alveolar wall.

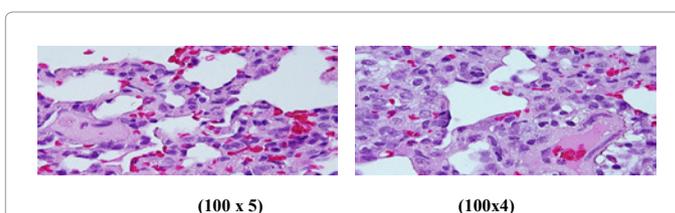


Figure 3: GNPs-treated rat received 100 µl of 10 nm particles for 7 days demonstrated diffuse interstitial tissue infiltrated by chronic inflammatory cells, scattered red blood cells, patent alveolar spaces, and congested and dilated blood vessels.

demonstrated inflammatory cells infiltrate with extravasation of red blood cells and with thickened alveolar wall as shown in Figure 4.

4) GNPs-treated rat receiving 100 µl of 20 nm particles for 7 days demonstrated diffuse interstitial tissue infiltrated by chronic inflammatory cells, scattered red blood cells, patent alveolar spaces, and congested and dilated blood vessels as shown in Figure 5.

5) GNPs-treated rat receiving 100 µl of 50 nm particles for 3 days demonstrated interstitial inflammatory cells infiltrate with small lymphocytes, plasma cells and considerable number of eosinophils, and surrounded by congested and dilated blood vessels with patent clear alveolar spaces as shown in Figure 6.

6) GNPs-treated rat receiving 100 µl of 50 nm particles for 7 days demonstrated dense interstitial inflammatory cells infiltrate of chronic type with patent clear alveolar space as shown in Figure 7.

The alterations induced by intraperitoneally administration of GNPs were size-dependent with smaller ones inducing more affects

and related with time exposure of GNPs. None of the above alterations were observed in the lung tissue of any member of the control group.

This infiltration was more prominent after 7 days of administration and in rats receiving 10 and 20 nm GNPs than those receiving 50 nm GNPs. The appearance of interstitial lung pneumonia with small lymphocytes, plasma cells and eosinophils, extravasation of red blood cells, and congested, thickened and dilated blood vessels may suggest that GNPs could interfere with the antioxidant defense mechanism, leading to reactive oxygen species (ROS) generation which in turn may imitate an inflammatory response. Inflammatory cells infiltration was seen in the portal triads and the perioral zones of GNPs treated rats. The infiltrate cells were mainly lymphocytes and plasma cells [12-15].

It has been reported that 5 nm GNPs caused significantly greater oxidative stress and cytotoxicity effects than larger ones [16,17]. The 5 nm GNPs have shown to catalyze nitric oxide (NO) production

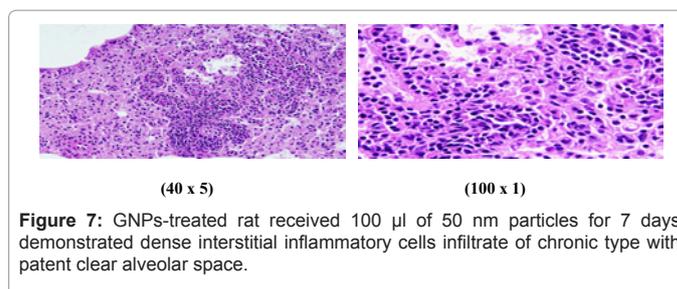


Figure 7: GNPs-treated rat received 100 μ l of 50 nm particles for 7 days demonstrated dense interstitial inflammatory cells infiltrate of chronic type with patent clear alveolar space.

from endogenous S-nitroso adducts with thiol groups in blood serum. NO reacts rapidly with superoxide producing peroxynitrite (ONOO⁻) which can interact with lipids, DNA, and proteins via direct oxidative reactions or via indirect radical-mediated damage [17]. ROS production could result from the proportionately high surface area of GNPs used in this investigation [18].

NPs are nearly of same dimensions as some biological molecules such as proteins and nucleic acids. Many of these biomolecules consist of long macromolecular chains which are folded and shaped by cooperative and weak interaction between side groups. The GNPs may intrude into these complex folded structures.

GNPs activate the phagocytic activity of the sinusoidal cells by increasing the number of Kupffer cells to help in removing the accumulated GNPs where lysosomes are involved in the intracellular breakdown into small metabolic products. Kupffer cell hyperplasia is contributed to hepatic oxidative stress [19].

Fatty change was observed in some swelling hepatocytes of rats exposed to 100 μ l of 10 nm GNPs and to lesser extent in the ones exposed to larger particles. This hepatic liposis was more prominent in rats exposed to GNPs for 7 days than those receiving the treatment for 3 days [12-15]. Hepatocytes fatty change might be due to lipid peroxidation that leads to rough endoplasmic damage.

The rats receiving 10 and 20 nm GNPs showed lung pneumonia with small lymphocytes, plasma cells and eosinophils, extravasation of red blood cells, alveolar spaces, and congestion and dilation of blood vessels of red blood cells. Less disruption was observed in rats exposed to 50 nm GNPs while more damage was detected after 7 days than 3 days of GNPs exposure. This alteration might indicate lung tissue damage and congestion by GNPs exposure.

The interaction of NPs with living systems is also affected by the characteristic dimensions. As noted above, GNPs, of smaller size may reach inside biomolecules, a situation not possible for larger GNPs. It has been reported that inhaled NPs reach the blood and may reach other target sites such as the liver, lung or blood cells [20,21].

The reduction in particle size results in an enormous increase of surface to volume ratio, so relatively more molecules of the chemical are present on the surface, thus enhancing the intrinsic toxicity [21]. This may be one of the reasons that smaller GNPs are generally more toxic than larger particles of the same insoluble material when compared on a mass dose base [22].

For several different NPs, only Co induced toxicity in endothelial cells, which was accompanied by the production of the pro-inflammatory cytokine IL8 [23]. The Cd, Ni and Pb concentrations significantly increased in blood and several tissues of rats after the intraperitoneal administration of 10, 20 and 50 nm GNPs compared

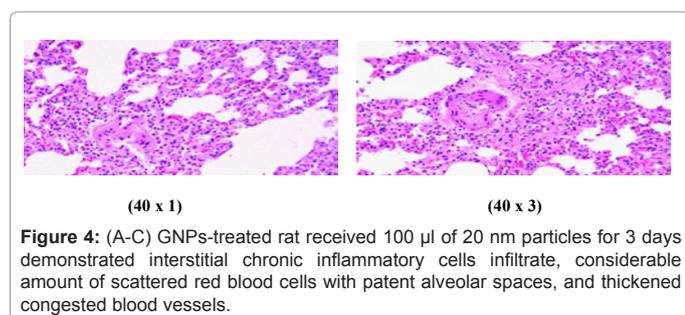


Figure 4: (A-C) GNPs-treated rat received 100 μ l of 20 nm particles for 3 days demonstrated interstitial chronic inflammatory cells infiltrate, considerable amount of scattered red blood cells with patent alveolar spaces, and thickened congested blood vessels.

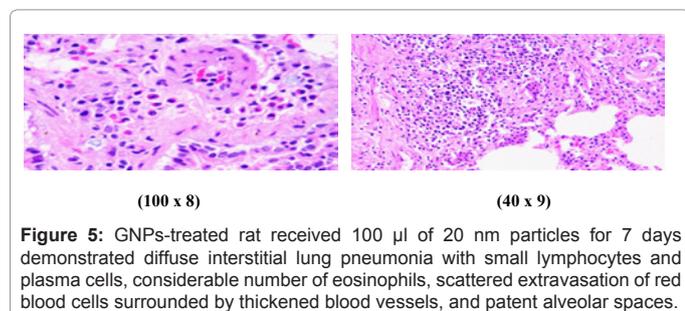


Figure 5: GNPs-treated rat received 100 μ l of 20 nm particles for 7 days demonstrated diffuse interstitial lung pneumonia with small lymphocytes and plasma cells, considerable number of eosinophils, scattered extravasation of red blood cells surrounded by thickened blood vessels, and patent alveolar spaces.

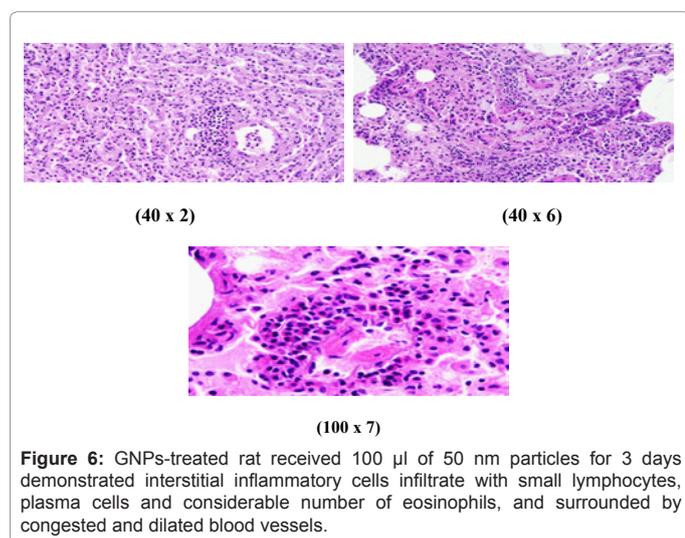


Figure 6: GNPs-treated rat received 100 μ l of 50 nm particles for 3 days demonstrated interstitial inflammatory cells infiltrate with small lymphocytes, plasma cells and considerable number of eosinophils, and surrounded by congested and dilated blood vessels.

with the control while different changes were observed with the Co concentrations [24].

The present study demonstrates that the inflammation produced in the lung tissue and other tissues/organs [12-15] was more prominent with smaller GNPs inducing more affects and related with time exposure of GNPs.

In present study we have not measured GNPs concentration in urine and feces, but this point will be taken into our consideration in other new additional experiments.

This study suggests that additional experiments related to oxidative stress in cells and organs, plasma, tissues cytokine are now taken in consideration and performed to cover and understand the toxicity induced by administration of GNPs.

Conclusions

Histological alterations induced in the lung tissue exposure as shown from the results of the present work can be summarized as interstitial lung pneumonia with small lymphocytes, plasma cells and considerable number of eosinophils, extravasation of red blood cells, and congested, thickened and dilated blood vessels. One might conclude that these alterations are size-dependent with smaller ones inducing more damage in relation with the time of exposure of GNPs.

Competing Interests

Author declares that he has no competing interests.

Author's contributions

Abdelhalim MAK has analyzed data, interpreted and written the final draft of this manuscript. The animal model used in this study was obtained from the Laboratory Animal Center (College of Pharmacy, King Saud University, Saudi Arabia). Abdelhalim MAK has conceived the study and its design and obtained research grants for this study. The authors have read and approved the final manuscript.

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