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Exposure to Gold Nanoparticles Produces Pneumonia, Fibrosis, Chronic Inflammatory Cell Infiltrates, Congested and Dilated Blood Vessels, and Hemosiderin Granule and Emphysema Foci

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Abstract

Background: For the application of gold nanoparticles (GNPs) in therapy and drug delivery, it is necessary to characterize histological lung tissue alterations after the administration of GNPs. These histological lung alterations have not been previously documented. The present study attempted to characterize histological lung tissue alterations after intraperitoneal administration of different GNP sizes to understand their bioaccumulation and toxicity role and determine whether these alterations are related to GNP size and exposure duration.

Methods: A total of 40 healthy male Wistar-Kyoto rats received 50 µl infusions of 10, 20, and 50 nm GNPs for 3 or 7 days. Animals were randomly divided into four groups: three GNP-treated groups and one control group. Groups 1, 2 and 3 received 50 µl infusions of 10, 20 and 50nm for 3 or 7 days GNPs, respectively.

Results: GNP-treated rats that received 50 µl of 10 and 20 nm particles for 3 or 7 days demonstrated more diffuse interstitial pneumonia, fibrosis, chronic inflammatory cell infiltrates of small lymphocytes, congested and dilated blood vessels, scattered dense extravasation of red blood cells, and foci of hemosiderin granules compared with rats that received 50 µl of 50 nm particles for 3 or 7 days.

Conclusions: These histological alterations are size-dependent; smaller particles induce more damage and are also related to the duration of exposure to GNPs. Histological lung tissue alterations are due to toxicity induced by exposure to GNPs; the tissue becomes unable to deal with the accumulated residues resulting from metabolic and structural disturbances caused by these particles. The histological lung alterations suggest that GNPs might interact with proteins and enzyme of the lung tissue, interfering with the antioxidant defense mechanism and leading to free radical and reactive oxygen species generation and lipid peroxidation.

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

Background

Nanoparticles (NPs) may differ in reactivity and solubility and may interact with all kinds of endogenous proteins, lipids, polysaccharides, and cells. Based on experiences in inhalation toxicology, a series of tests was proposed for evaluation of the toxicity of NPs used in drug delivery systems [1]. Gold NPs (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 [2,3].

Gold in its bulk form has been considered an inert, noble metal with some therapeutic and medicinal value. GNPs are thought to be relatively non-cytotoxic [4], while the metallic nature of metal-derived NPs and the presence of transition metals encourages the production of reactive oxygen species (ROS), leading to oxidative stress [5,6]. There are differing reports of the extent of the toxic nature of these particles owing to the different modifications of GNPs, surface functional attachments, and shape and diameter of the NPs [7,8].

The particle size-dependent organ distribution of GNPs has been studied *in vivo* [9-11]. *In vivo* studies of 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 [12].

To understand and categorize the mechanisms of GNP 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, which implies an urgent need for histological data related to GNPs.

Lung tissue histological and histochemical alterations due to the administration of GNPs have not been previously documented. In the present study, an attempt was made to characterize lung tissue histological alterations after intraperitoneal administration of GNPs of various sizes and determine whether these alterations are related to GNP size and exposure duration.

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). All rats were nearly the same age (12 weeks old), weighed 220 to 240 g, and were from the King Saud University

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colony. Animals were randomly divided into four groups: three GNP-treated groups and one control group. The 10, 20, and 50 nm GNPs were administered intraperitoneally at the rate of 3 or 7 days as follows: Group 1 received 50 µl infusions of 10 nm GNPs for 3 or 7 days (n = 10); Group 2 received 50 µl infusions of 20 nm GNPs for 3 or 7 days (n = 10); Group 3 received 50 µl infusions of 50 nm GNPs for 3 or 7 days (n =10); and the 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 the 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%), and dehydrated with varying grades of ethanol (70%, 80%, 90%, 95%, and 100%). Dehydration was followed by clearing the samples in two changes of xylene. Samples were then impregnated with two changes of molten paraffin wax, embedded, and blocked out. Paraffin sections (4–5 um) were stained with hematoxylin and eosin (the conventional histological stain) according to Pearse [13]. 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 lung tissues were examined for histological alterations.

Results and Discussion

Size and morphology of different GNPs

The 10 and 20 nm GNPs had a spherical shape, while the 50 nm GNPs had a hexagonal shape. The mean size of the GNPs was calculated from the images taken by a transmission electron microscope: The mean size of the 10 nm GNPs was 9.45 ± 1.33 nm, that of the 20 nm GNPs was 20.18 ± 1.80 nm, and that of the 50 nm GNPs was 50.73 ± 3.58 nm [14-17].

Histological alterations

No mortality occurred during the GNP administration periods of 3 and 7 days in any of the experimental groups of the present investigation, and no alterations were observed in the appearance or behavior of GNP-treated rats compared with the control rats.

In this study we would like to shed the light on the side effects induced by intraperitoneal administration of GNPs because these effects especially in the lung tissue have not been documented before.

Control group (Figure 1): Microscopic pictures show GNP-normal rat demonstrating well-formed and open alveoli with normal septae, few scattered small lymphocytes, and minimal eosinophils.

In this study we inserted only the lung images which indicate clear histopathological effects regarding the magnification effects. Compared with the control group, the following histological alterations were detected in the lung tissue of GNP-treated rats. These histological alterations were observed in Figures 2-7. The histological alterations can be summarized as follows:

The tissue alterations induced by intraperitoneal administration of GNPs were size-dependent; smaller GNPs induced more effects, and these tissue alterations were related to GNP exposure duration. Abdelhalim and Jarrar [17] has reported that the interaction of GNPs with proteins and various cell types might be evaluated as part of the toxicological assessment in addition to further experiments related to tissues antioxidant enzymes, oxidative parameters, lipid peroxidation, production of free radicals and/or ROS and cytokine will be performed before using the GNPs as therapeutic and diagnostic tool.

This infiltration was more prominent after 7 days of administration and in rats that received 10 and 20 nm GNPs than in those that received 50 nm GNPs. The appearance of lymphocytic infiltrate with extravasation of red blood cells in the lung tissue may suggest that GNPs could interfere with the antioxidant defense mechanism and

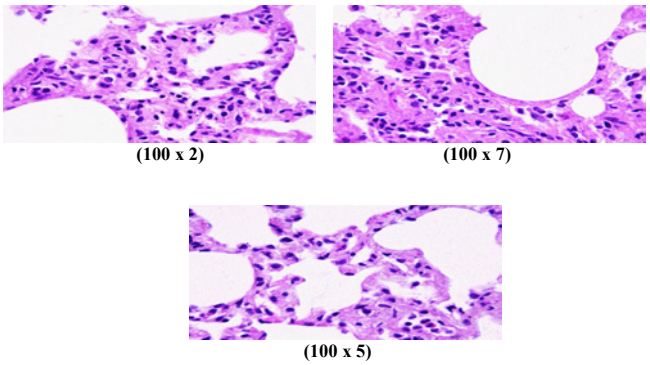

Figure 1: GNPs-normal rat demonstrating well-formed and opened alveoli with normal appearing septum, few scattered small lymphocytes, minimal eosinophils.

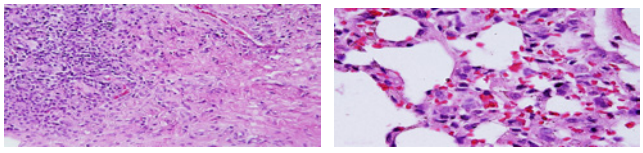

Figure 2: GNPs-treated rat received 50 µl of 10 nm particles for 3 days demonstrating more diffuse interstitial lung pneumonia, dense inflammatory cells infiltrate of small lymphocytes, fibrosis and more prominent extravasation of red blood cells.

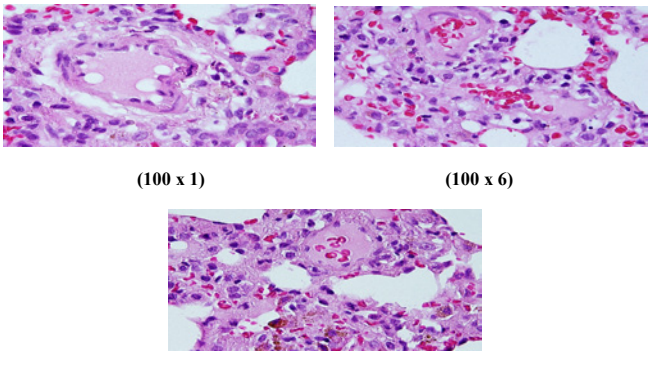

Figure 3: GNPs-treated rat received 50 µl of 10 nm particles for 7 days demonstrating prominent chronic inflammatory cells infiltrate surrounding by dilated and congested blood vessels, scattered dense extravasation of red blood cells and foci of hemosiderin granules.

lead to ROS generation, which in turn may imitate an inflammatory response. Inflammatory cell infiltration was seen in the portal triads and perioral zones of GNP-treated rats. The infiltrate cells were mainly lymphocytes and plasma cells [14-17].

GNPs were more strongly oxidizing as evidenced by lipid peroxidation [18,19] and decreased neutral red retention time and numbers of thiol-containing proteins evident in electrophoretic separations. Cadmium may displace iron or copper from metalloproteins, leading to oxidative stress via the Fenton reaction [20].

It has been reported that 5 nm GNPs cause significantly greater oxidative stress and cytotoxicity effects than do larger ones [21-23]. The 5 nm GNPs have been shown to catalyze nitric oxide (NO) production from endogenous S-nitroso adducts with thiol groups in blood

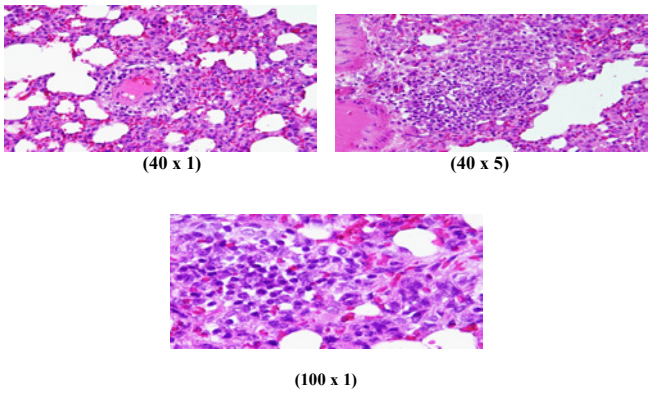

Figure 4: GNPs-treated rat received 50 µl of 20 nm particles for 3 days demonstrating dense interstitial inflammatory cells infiltrate, congested and dilated blood vessels, and scattered extravasation of red blood cells.

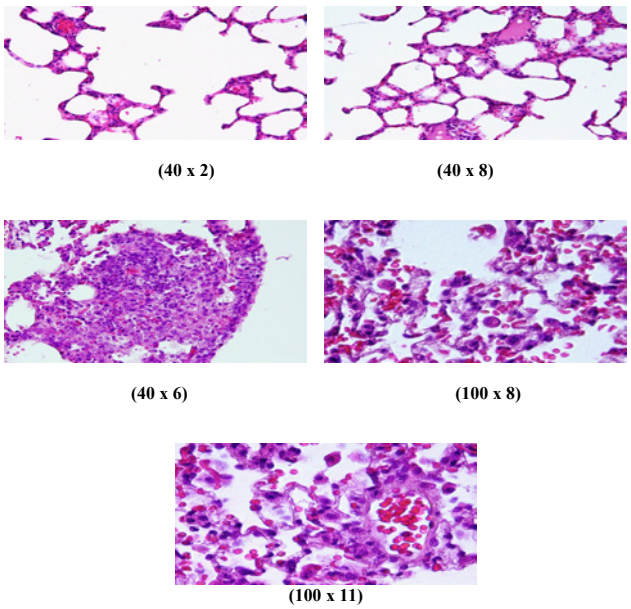

Figure 5: GNPs-treated rat received 50 µl of 20 nm particles for 7 days demonstrating thin, dilated and disconnected alveolar wall end by knob formation, prominent focus of emphysema, interstitial dense inflammatory cells infiltrate with small lymphocytes, macrophages and scattered red blood cells, and congested and dilated blood vessels.

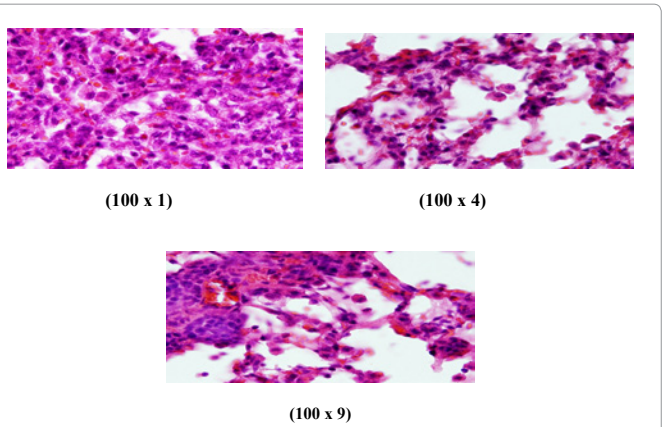

Figure 6: GNPs-treated rat received 50 µl of 50 nm particles for 3 days; demonstrating more prominent pneumonic patches, interstitial inflammatory cells infiltrate, extravasation of red blood cells, inflammatory cells infiltrate affecting the alveolar septum.

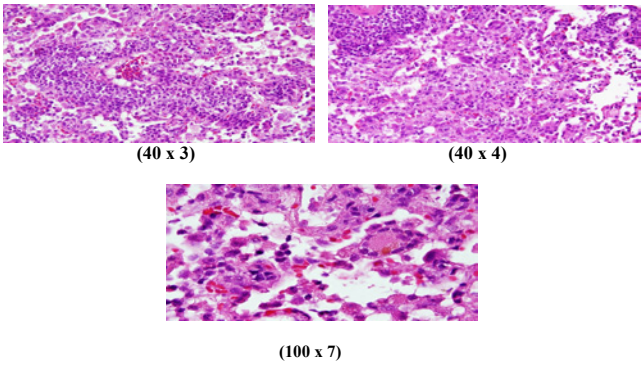

Figure 7: GNPs-treated rat received 50 µl of 50 nm particles for 7 days demonstrating more prominent features of lung pneumonia with diffuse interstitial and intra alveolar dense inflammatory cells infiltrate, and extravasation of red blood cells associated with one focus of foreign body giant cell and surrounded by inflammatory cells.

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 [24]. ROS production could result from the proportionately high surface area of GNPs used in this investigation [25,26].

GNPs have dimensions nearly identical to those of some biological molecules, such as proteins and nucleic acids. Many of these biomolecules consist of long macromolecular chains that are folded and shaped by cooperative and weak interactions between side groups. The GNPs may intrude into these complex folded structures.

GNPs activate the phagocytic activity of sinusoidal cells by increasing the number of Kupffer cells and helping to remove the accumulated GNPs, where lysosomes are involved in intracellular breakdown into small metabolic products. The produced Kupffer cell hyperplasia might be correlated with the amount of injury to the hepatic tissue induced by GNP intoxication and represents a defense mechanism of detoxification. Kupffer cell hyperplasia is contributed to hepatic oxidative stress [27]. The rats that received 10 and 20 nm GNPs showed more disruption in the lung tissues than did those exposed to 50 nm GNPs, while more damage was detected after 7 days than

3 days of GNP exposure. This alteration might indicate lung damage and congestion by GNP exposure. None of the above alterations were observed in the lung tissue of any member of the control group.

The interaction of NPs with living systems is also affected by the characteristic dimensions of the NPs. As noted above, smaller GNPs 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 [28,29].

Reduction in size results in an enormous increase of surface-to-volume ratio; thus, relatively more molecules of the chemical are present on the surface, enhancing the intrinsic toxicity [30]. This may be one of the reasons why smaller GNPs are generally more toxic than larger particles of the same insoluble material when compared on a mass dose basis [31].

Among several different NPs, only Co induced toxicity in endothelial cells, which was accompanied by the production of the pro-inflammatory cytokine IL8 [32]. The Cd, Ni, and Pb concentrations significantly increased in blood and several tissues of rats after intraperitoneal administration of 10, 20, and 50 nm GNPs compared with the control, while different changes were observed with Co concentrations [33].

The present study demonstrates that the inflammation produced in the lung tissue and other tissues or organs [20-23] was more prominent with smaller GNPs, which induced more effects that were also related to GNP exposure duration.

GNP concentrations in tissues, urine and feces were not measured in the present study, but this point will be taken into consideration in additional experiments.

Images can be analysed by histopathology analysis software's to quantitate the amount of damage caused due to nanoparticle based on surface area ratio of the images. Then, a histogram can be plotted to clearly illustrate the amount of damage caused in various concentrations of nanoparticle treatments to rats. To achieve this point we require quantifying the lung images under the action of same image magnification, area, GNPs dose, exposure duration, and size. Thus, another manuscript will be prepared taking this point in consideration.

This study suggests that one possible way of avoiding significant toxicity is to coat the GNPs with biocompatible polymers. Now days other experiments to effectively demonstrate and avoid the possible side effects of GNPs in the *in vivo* studies will be taken in consideration and conducted.

Conclusions

Compared with the respective control rats, the histological alterations can be summarized as follows: GNP-treated rats that received 50 µl of 10 and 20 nm particles for 3 or 7 days demonstrated more diffuse interstitial pneumonia, fibrosis, chronic inflammatory cell infiltrates of small lymphocytes, congested and dilated blood vessels, scattered dense extravasation of red blood cells, and foci of hemosiderin granules compared with GNP-treated rats that received 50 µl of 50 nm particles for 3 or 7 days.

These alterations appear to be size-dependent; smaller ones induced more damage in relation to GNP exposure duration.

Histological lung tissue alterations might be due to the accumulated residues from metabolic and structural disturbances caused by these particles.

One mechanism of NP toxicity is likely the production of ROS and the consequential oxidative stress in cells and organs. The lung tissue histological alterations suggest that GNPs might interact with proteins and enzymes of the lung tissue, interfering with the antioxidant defense mechanism and leading to ROS generation, which in turn may induce stress in the lung tissue.

Further experiments that take into consideration the interaction of GNPs with proteins, various cell types, antioxidant enzymes, tissue cytokines, and lipid peroxidation will be performed to understand the potential therapeutic and diagnostic applications of GNPs.

Competing Interests

The author declares no competing interests.

Author's Contributions

AMAK analyzed and interpreted the data and wrote 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). AMAK conceived the study and its design and obtained research grants for this study. The author has read and approved the final manuscript.

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