Neurotoxicology and Teratology 51 (2015) 68–76

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Systemic and behavioral effects of intranasal administration of silver nanoparticles

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ARTICLE INFO

Article history:
Received 8 January 2015
Received in revised form 17 July 2015
Accepted 25 August 2015
Available online 2 September 2015

Keywords:
Silver nanoparticles
Neurobehavioral effects
Autometallography
Intranasal instillation
Morris Water Maze

ABSTRACT

Use of silver nanoparticles (AgNPs) for their antimicrobial properties is widespread. Much of the previous work on the toxicity of AgNPs has been conducted in vitro or following oral or intravenous administration in vivo. Intranasal (IN) instillation of AgNPs mimics inhalation exposure and allows further exploration of the toxicity of these particles via respiratory tract exposure. The present study involved 1) single-dose exposures to assess tissue distribution and toxicity and 2) repeated exposures to assess behavioral effects of IN AgNP exposure (nominally uncoated 25 nm AgNP). AgNP deposition was localized in the liver, gut-associated lymphoid tissue, and brain. Decrease cellularity in spleen follicles was observed in treated mice, along with changes in cell number and populations in the spleen. The splenic GSH:GSSG ratio was also reduced following AgNP exposure. Expression of the oxidative stress-responsive gene *Hmox1* was elevated in the hippocampus, but not cortex of treated mice, as was the level of HMOX1 protein. Mice receiving 7 days of IN exposure to 50 mg/kg AgNPs exhibited similar learning- and memory-related behaviors to control mice, except that treated mice spent significantly less time in the target quadrant of the Morris Water Maze during the acquisition phase probe trial. These findings indicate systemic distribution and toxicity following IN administration of AgNPs.

1. Introduction

Use of silver nanoparticles (AgNPs) in consumer goods has become increasingly prevalent due to their antimicrobial properties. Textiles, aerosol sprays, water purification systems, and coatings on medical implants and instruments may contain AgNPs (Seltenrich, 2013; Chen and Schluesener, 2008; U.S. EPA, 2012). Despite their prevalence, little is known about the risks of inhalation exposure for manufacturing employees or consumers. It is estimated that between 2.8 and 20 t of nanosilver are produced each year in the United States (Hendren et al., 2011). Lee et al. (2012) demonstrated that employees are exposed to these particles during production.

Previous research with AgNPs has illustrated their ability to cause damage to various types of tissue throughout the body. Oral administration of AgNPs was shown to cause hepatotoxicity and systemic inflammation (Park et al., 2010). Intraperitoneal (IP) injection of AgNPs led to changes in oxidative stress-mediated gene expression in the brain (Rahman et al., 2009). Sung et al. (2008) found decreased pulmonary function and lung inflammation in rats exposed by inhalation to AgNPs. We previously showed the ability of AgNPs to distribute throughout the body following intranasal (IN) exposure, including to the brain, spleen, lungs, and kidneys (Genter et al., 2012). Administration of particles via the IN route mimics exposure via inhalation through the nose, the most likely exposure route for manufacturing workers.

The present study was performed to expand the range of IN doses of AgNPs from our previous study and to explore additional endpoints. These additional endpoints included evaluation of additional tissues (liver and gut-associated lymphoid tissues), as well as more extensive evaluation of the responses of the spleen and brain. Our previous work and that of others suggest that silver nanoparticles can cause oxidative stress (Genter et al., 2012; Rahman et al., 2009). Therefore, changes in cell number and populations and glutathione status were evaluated in the spleen, as well as brain regions for oxidative stress markers, were examined. Increased expression of the oxidative stress

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http://dx.doi.org/10.1016/j.ntt.2015.08.006
0892-0362/

responsive gene heme oxygenase (*Hmox1*; Keyse and Tyrrell, 1989), suggesting particle-induced oxidative stress in the hippocampus of treated mice, also led to exploration of the effects of IN AgNP administration on learning and memory.

2. Materials and methods

2.1. Reagents

The silver nanoparticles under investigation in this study were nominally 25 nm uncoated particles supplied by Dr. Syed Ali (National Center for Toxicological Research, Jefferson, AR) and originally obtained from NovaCentrix (Austin, TX, USA).

2.2. Transmission electron microscopy (TEM)

Nanoparticles were subjected to TEM in the Advanced Materials Characterization Center at the University of Cincinnati. AgNPs were suspended in ethanol and deposited on a TEM grid and dried before analysis. An FEI CM-20 microscope operated at 200 Kv was used to observe particle diameter and distribution. Mean AgNP particle diameter was found to be 39.97 ± 17.98 nm (Fig. 1). These particles were previously characterized by Rahman et al. (2009), with DLS results showing the particles 118 nm in DI water and at 1090 nm in PBS. These authors also showed a zeta potential value of −34.6 mV.

2.3. Study design

The present study was conducted in two parts:

single-dose IN exposures (10–500 mg/kg) were conducted at doses of 10–500 mg/kg, and the mice euthanized days later. These doses were selected to examine the effects of doses lower than those in our previous publication (Genter et al., 2012), while keeping the highest dose previously used as an internal control. No behavioral analysis was performed on these mice; and

a repeat-dose study was performed (50 mg/kg/day for 7 days, followed by a 7-day wait period) for neurobehavioral assessment.

2.4. Mouse treatments

Male C57BL/6 mice (Charles River Laboratories, Wilmington, MA for behavior studies, Jackson Laboratory, Bar Harbor, ME for all other studies) were acclimated for one week following arrival. Mice were housed two to four per cage and provided water and pelleted chow *ad libitum*. Housing was under consistent environmental conditions (50% humidity, 12 h light–dark cycle, 22 ± 1 °C). Particles were suspended in sterile ultrapure water, sonicated for 10 s, and vortexed immediately prior to instillation. The AgNP dosing suspension (50–62.5 mg/mL, depending on the body weight of the mice), or sterile water for control, was delivered in a volume of 10 μL per nostril via sterile pipette tip, as previously described (Genter et al., 2012). Mice were cared for in accordance with federal animal care guidelines. This research was approved by the University of Cincinnati Institutional Animal Care and Use Committee and was performed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility.

2.5. Evaluation of endpoints after single-dose treatments

Histology and autometallography (AMG): At necropsy, tissues were collected and put into formalin (e.g. brain, kidney, liver, spleen, and gut-associated lymphoid tissue (GALT)) for histological analysis. Tissues were then paraffin-embedded, sectioned at 5 μm, and subjected to hematoxylin and eosin (H&E) staining or AMG. AMG is an established method for the detection of metal colloid aggregates in histological sections (Danscher and Stoltenberg, 2006) and was previously used for localization of AgNP deposition in our laboratory (Genter et al., 2012). Briefly, deparaffinized sections were incubated at 30 °C for 90 min in a solution containing 1.2% carboxymethylcellulose (*w*/*v*) (Sigma-Aldrich, St. Louis, MO), citrate buffer solution (1.2 M citric acid + 0.8 M sodium citrate, pH 3.7), 8.6% hydroquinone (*w*/*v*), and 0.12% silver lactate (*w*/*v*) (Acros Chemicals, Brunswick, NJ) in the dark. Following a wash step, slides were incubated in a 5% sodium thiosulfate solution at RT

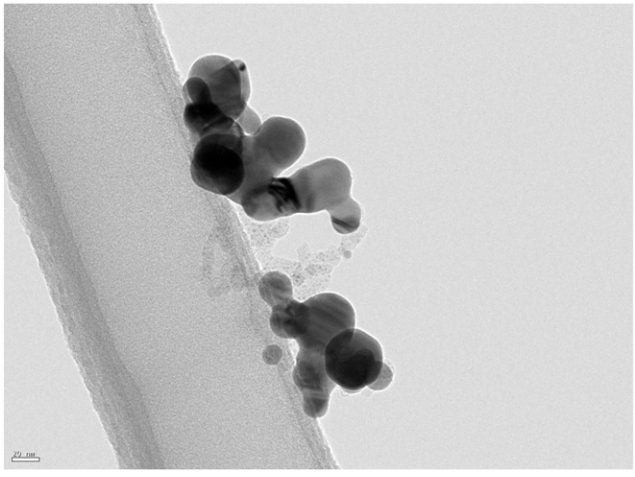

Fig. 1. Representative TEM images of the particles used in these studies. Approximately 200 particles were sized, with a mean value of 39.97 ± 17.98 nm in diameter. Bar = 20 nm.

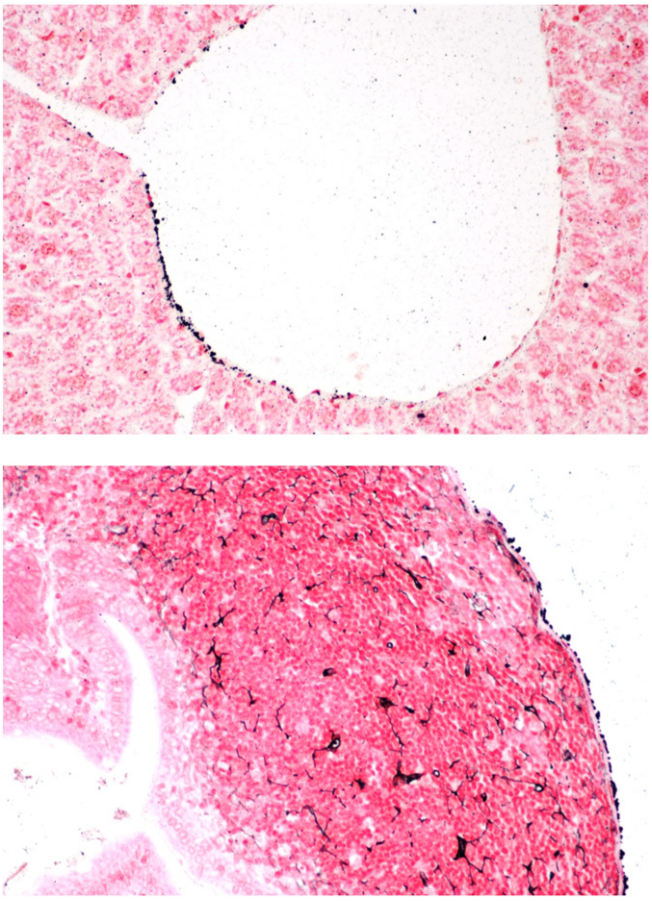

Fig. 2. Autometallography showing silver deposition in peripheral tissues 7 days after IN administration of single doses of AgNP. Black staining is indicative of the presence of AgNP aggregates. Top panel: central vein of liver from a mouse treated with 10 mg/kg; bottom panel: gut associated lymphoid tissue from a mouse treated with 500 mg/kg. No black depositions were found in tissues from vehicle-treated controls in these tissues.