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Acute intravenous exposure to silver nanoparticles during pregnancy induces particle size and vehicle dependent changes in vascular tissue contractility in Sprague Dawley rats

A.K. Vidanapathirana[a], L.C. Thompson[a], M. Herco[a], J. Odom[a], S.J. Sumner[b,d], T.R. Fennell[b], J.M. Brown[c], C.J. Wingard[a,e,*]

[a] Department of Physiology, Brody School of Medicine, East Carolina University, Greenville, NC, 27834, USA
[b] Discovery Sciences, RTI International, Research Triangle Park, NC, 27709, USA
[c] Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado, CO, 80045, USA
[d] Department of Nutrition School of Public Health University of North Carolina at Chapel Hill, Kannapolis, NC, 28081, USA
[e] Department of Physical Therapy, Bellarmine University, Louisville, KY, 40205, USA

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ABSTRACT

The use of silver nanoparticles (AgNP) raises safety concerns during susceptible life stages such as pregnancy. We hypothesized that acute intravenous exposure to AgNP during late stages of pregnancy will increase vascular tissue contractility, potentially contributing to alterations in fetal growth. Sprague Dawley rats were exposed to a single dose of PVP or Citrate stabilized 20 or 110 nm AgNP (700 μg/kg). Differential vascular responses and EC_{50} values were observed in myographic studies in uterine, mesenteric arteries and thoracic aortic segments, 24 h post-exposure. Reciprocal responses were observed in aortic and uterine vessels following PVP stabilized AgNP with an increased force of contraction in uterine artery and increased relaxation responses in aorta. Citrate stabilized AgNP exposure increased contractile force in both uterine and aortic vessels. Intravenous AgNP exposure during pregnancy displayed particle size and vehicle dependent moderate changes in vascular tissue contractility, potentially influencing fetal blood supply.

1. Introduction

Engineered silver nanoparticles (AgNP) are increasingly used in many consumer applications including food, clothing and infant products [1,2]. They have also been developed and used in various biomedical applications including intravenous (IV) catheters, orthopedic bone cement, surgical mesh, vascular stents and wound dressings [3]. The inherent antimicrobial, antiviral and anti-inflammatory properties of AgNP underlies their utility in medical [3] and household products [1,2]. However, some applications may lead to increased exposure followed by distribution and/or accumulation within the human body, raising concerns regarding their safety, particularly in susceptible life stages such as pregnancy, which may affect intrauterine fetal growth.

Silver nanoparticles are engineered in various sizes or forms and formulated with various carrier molecules to increase their stability, biocompatibility and versatility. As a result, any differential tissue accumulation/distribution by varying size or coatings of AgNP [4,5], and the resultant silver ion dissolution or intracellular aggregation/precipitation [5–10] may contribute to differential tissue responses.

Inhalational exposure to AgNP [9–13] suggests cellular inflammatory responses with persistent silver accumulation in immune cell or surrounding tissue, but were not examined for impact on life stage. However, changes in vascular tissue responses are reported with other metal based (nickel hydroxide and nano-TiO_2) nanoparticle exposure studies in both pregnant [14] and

Abbreviations: 5HT, serotonin; Ach, acetylcholine; AgNP, silver nanoparticles; AgNP/Citrate, citrate-stabilized nanosilver particle; AgNP/PVP, polyvinylpyrorrolidone-stabilized nanosilver particle; ANG II, angiotensin II; DLS, dynamic light scattering; ENM, engineered nanomaterials; ET-1, endothelin 1; EC_{50}, Effective Concentration for 50% of effect; GD, gestational day; IV, intravenous; NCNHIR, National Institute of Environmental Health Sciences Centers for Nanotechnology Health Implications Research; MA, mesenteric artery; MUA, main uterine artery; NP, non-pregnant; P, pregnant; PE, phenylephrine; PSS, physiological saline solution; PVP, polyvinylpyrorrolidone; SEM, standard error of the mean; TA, thoracic aorta; TEM, transmission electron microscopy.

* Corresponding author at: Department of Physical Therapy, College of Health Professions, Bellarmine University, 2001 Newburg Rd, Louisville, KY 40205, USA.
E-mail address: cwingard@bellarmine.edu (C.J. Wingard).

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non-pregnant [15,16] life stages. In addition, aerosol exposure to nano-sized titanium dioxide during gestation negatively impacted cardiac function, mitochondrial respiration and bioenergetics in the progeny [17]. The direct *in vitro* exposure of human bronchial epithelial cells to PVP coated AgNP was reported to induce DNA damage as assessed by the comet assay [18], suggesting that *in vivo* exposure may also be associated with detrimental changes in the target tissue or organ function. Current evidence on AgNP distribution suggests that following oral/intragastric delivery, AgNP or silver ion can be distributed to different organs including liver and kidneys through absorption from the vascular compartment [19,20], as well as accumulation in the placenta and fetuses during pregnancy [21]. However, consensus on overt toxicity from these studies is far from clear as it is suggested that the form of silver within the biological medium may be important in rendering any effect [20,22–26].

There are reported gender related differences in the distribution and biokinetics of intravenously administered 15 nm AgNP [27]. The distribution in blood, liver and bone marrow of AgNP following intravenous injection in a mouse model, revealed cytotoxic and oxidative stress responses that varied with the dose, size and coating on AgNP [28]. The reported distribution of various sized AgNP during pregnancy are mainly to maternal organs including liver, spleen and lung following IV exposure [29]. Silver particles were detected in the fetal yolk sac, suggesting potential distribution in the fetal circulation affecting fetal growth and development. In addition, other studies with intravenous silica and titanium nanoparticle exposures during pregnancy have identified fetal abnormalities [30]. Recent studies with intravenously administered PVP formulated 20 nm and 110 nm AgNP have shown particle size dependent maternal deposition in the spleen/liver as well as feto-maternal transfer. While silver was detected in fetuses, relatively higher concentrations were detected in the placentae [21]. Largely, reproductive and developmental toxicity effects have been reported in many studies via different routes of maternal exposure to various forms of AgNP resulting in differential tissue distribution [21,26,31] all possibly trafficking through the vascular system.

During the distribution and excretion process, the vascular endothelium and underlying smooth muscle are potential targets of the silver exposure. The *ex vivo* exposure to 45 nm AgNP has been reported to increase vasoconstriction in isolated male rat aortic rings and *in vitro* exposure is associated with alterations in the availability of nitric oxide in coronary endothelial cells [32]. The reported AgNP mediated changes of vascular tissue contractile properties and alterations in the uterine blood supply during gestation would suggest a potential negative affect on fetal development. Previous studies from our laboratory with intravenous exposure to fullerenes (C60) and multi-walled carbon nanotubes (MWCNT) have reported nanoparticle and vehicle/suspension medium dependent changes in vascular tissue contractility in pregnancy, that may impact fetal growth [33,34].

The current study focuses on such changes with AgNP exposure, a nanoparticle with physicochemical properties different from carbon based nanotubes, but with reported differential effects on cardiac injuries studied previously via pulmonary exposure [35]. We hypothesized, intravenous exposure to AgNP during pregnancy will increase vascular tissue contractility, potentially contributing to a reduction in fetal growth. Variable contraction/relaxation effects between the uterine artery, mesenteric artery and thoracic aorta would accompany this response, dependent on the size and formulation of AgNP. We used two AgNP formulations, stabilized with PVP or citrate, of either 20 nm or 110 nm diameter size in an effort to elucidate any size and/or stabilization-vehicle dependent effects of commonly formulated AgNP on blood vessel responses following an acute exposure during pregnancy.

2. Methods

2.1. Silver nanoparticle formulations for exposure

20 and 110 nm spherical AgNP stabilized in citrate or polyvinylpyrrolidone (PVP), as produced by NanoComposix (San Diego, CA), were procured through the NIEHS Centers for Nanotechnology Health Implications (NCNHIR). Additional, independent characterization of the materials was performed by The Nanotechnology Characterization Laboratory (SAIC-Fredrick, Frederick, MD) [4,36]. Average values from that characterization including: silver content, core diameter by transmission electron microcopy (TEM), hydrodynamic diameter by dynamic light scattering (DLS), zeta potential and endotoxin content are reported in Table 1. The vehicle controls (stabilizing agents) were 2 mM citrate buffer or polyvinylpyrrolidone (pH 7.5), at the same concentration used for the AgNP suspensions, so that effects of the stabliizers could be separated from AgNP effects. Citrate buffer was prepared using sodium citrate (Sigma-Aldrich, St Louis, MO) in endotoxin free ultra-pure distilled water [37]. Four colloidal silver nanoparticle formulations were used for intravenous (IV) exposure studies, and will be referred to as indicated in parentheses; 20 nm, citrate-stabilized colloidal silver (20 nm AgNP/citrate), 20 nm, PVP (polyvinylpyrorrolidone)-stabilized colloidal silver (20 nm AgNP/PVP), 110 nm, citrate-stabilized colloidal silver (110 nm AgNP/citrate) and 110 nm, PVP-stabilized colloidal silver (110 nm AgNP/PVP). The citrate-stabilized particles were supplied in 2 mM citrate buffer and the PVP-stabilized particles were supplied in ultra- pure water with 1.4% PVP and a silver concentration of 1 mg/ml, in sealed 50 ml aliquots. The detailed characterization of 10, 25 and 50 ml aliquots of these particle suspensions were completed by the Nanotechnology Characterization Laboratory, National Cancer Institute at Frederick (SAIC-Frederick, Inc., Frederick, MD, USA) as reported in the Initial Characterization Data for NCNHIR Silver ENMs [36,38]. The size and shape of the AgNP were confirmed via transmission electron microscopy (TEM, Hitachi H7600). Size distribution analysis was performed using the freeware software Image J. A minimum of 100 particles per sample were counted by randomly surveying the entire TEM grid from multiple high magnification images. Image J was used to determine both area and Feret diameters [38]. Prior to their use, all suspensions were sonicated three times for 15 s using a Misonix ultrasonic liquid processor at 65% amplitude at a total energy output of 10,817 J (Model 1510R-MTH, Branson Ultrasonics Corp. Danbury, CT, USA). The silver suspensions were vortexed for 10 s in between sonication and prior to administration. These suspensions were stored at 4 °C in the dark between exposures and all particle handling procedures were done in a biological safety cabinet to ensure sterility.

2.2. Sprague dawley rats

Timed pregnant female Sprague Dawley rats, 10–12 weeks of age were purchased from Charles River Laboratories (USA). The rats arrived in the East Carolina University (ECU) Department of Comparative Medicine's animal facility between 9 and 12 days of gestation (GD). The exact GD was provided by the vendor for each dam on its arrival. Animals were allowed to acclimate for one week, housed individually, under 12 h light/dark cycles with standard rat chow and water provided *ad libitum* prior to exposure to the AgNP suspension. The body weight was monitored every three days to assess the progression of pregnancy. All animal handling, exposure