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Influence of physicochemical properties of silver nanoparticles on mast cell activation and degranulation

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ABSTRACT

Silver nanoparticles (AgNPs) are increasingly being incorporated into products for their antimicrobial properties. This has resulted in increased human exposures and the possibility of adverse health effects. Mast cells orchestrate allergic immune responses through degranulation and release of pre-formed mediators. Little data exists on understanding interactions of AgNPs with mast cells and the properties that influence activation and degranulation. Using bone marrow-derived mast cells and AgNPs of varying physicochemical properties we tested the hypothesis that AgNP physicochemical properties influence mast cell degranulation and osteopontin production. AgNPs evaluated included spherical 20 nm and 110 nm suspended in either polyvinylpyrrolidone (PVP) or citrate, Ag plates suspended in PVP of diameters between 40–60 nm or 100–130 nm, and Ag nanowires suspended in PVP with thicknesses <100 nm and length up to 2 μm. Mast cell responses were found to be dependent on the physicochemical properties of the AgNP. Further, we determined a role for scavenger receptor B1 in AgNP-induced mast cell responses. Mast cell degranulation was not dependent on AgNP dissolution but was prevented by tyrosine kinase inhibitor pretreatment. This study suggests that exposure to AgNPs may elicit adverse mast cell responses that could contribute to the initiation or exacerbation of allergic disease.

1. Introduction

The applications of nanotechnology are rapidly expanding and revolutionizing many fields primarily through the incorporation of nanoparticles (NPs) into numerous biomedical and consumer products. In particular, silver nanoparticles (AgNPs) are one of the most utilized NPs due to their antimicrobial/fungal properties (Dong et al., 2012; Levard et al., 2013; Nocchetti et al., 2013). More than 300 globally available consumer products, such as wound dressings, IV bags, dermal creams, water filters, and many household products, incorporate AgNPs (Project, 2014). Indeed, the annual global production of AgNPs is estimated to be >55 tons (Piccinno et al., 2012). A direct interaction of end user and AgNP-based products increases the risk of possible exposure through Ag or Ag^+ leaching out from these products and could possibly result in adverse health outcomes (Christensen et al., 2010). For instance, AgNPs used as coatings on surgical implants may enter into the systemic circulation and translocate into different organs such as the lung and/or liver (Rahman et al., 2009; Tang et al., 2009). More importantly, some food storage containers that use AgNP coatings that have been found to release nanostructured Ag into food due to an increase dissolution under high salt concentration (Echegoyen and Nerín, 2013). Animal studies have demonstrated that AgNP exposure results in hepatotoxicity and pulmonary inflammation (Sung et al., 2008; Tiwari et al., 2011). In addition, AgNPs have been reported to interact with immune cells and induce cytotoxicity through the generation of reactive

Abbreviations: AgNPs, silver nanoparticles; Blt2, blocking lipid transporter-2 (scavenger receptor B1 inhibitor); BMMC, bone marrow-derived mast cells; C110, 110 nm spherical silver nanoparticles suspended in citrate; C20, 20 nm spherical silver nanoparticles suspended in citrate; Lamp2, lysosome-associated membrane proteins 2; NPs, nanoparticles; OPN, osteopontin; P110, 110 nm spherical silver nanoparticles suspended in polyvinylpyrrolidone; P20, 20 nm spherical silver nanoparticles suspended in polyvinylpyrrolidone; P550, nanoplates with optical resonance peak at specific wavelengths of 550 nm suspended in polyvinylpyrrolidone; P850, nanoplates with optical resonance peak at specific wavelengths of 850 nm suspended in polyvinylpyrrolidone; PVP, polyvinylpyrrolidone; SR-B1, scavenger receptor B1.

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oxygen species (Carlson et al., 2008; Nishanth et al., 2011). To date, limited research exists evaluating the ability of NPs to directly interact with immune cells involved in allergy such as mast cells and possibly resulting in or exacerbation of allergic disease.

Mast cells are found in most tissue types and play an important role in innate immunity, host defense and allergic disease (Brown et al., 2008). Mast cells are well studied for their role in allergic disease and activation through IgE and the high affinity IgE receptor (FcεRI) leading to the release of a variety of mediators including histamine, serotonin, and inflammatory cytokines such as TNF-α, osteopontin (OPN), and eosinophil chemoattractant factor as examples (Brown et al., 2008). In addition, mast cells recognize pathogens through toll-like receptors and scavenger receptors (McCurdy et al., 2003; Medic et al., 2008). Recent animal studies have demonstrated that mast cells contribute to the inflammatory response following NP exposures. Specifically, it has been reported that mast cells are involved in lung inflammation and fibrosis following exposure to multi-walled carbon nanotubes (MWCNTs) (Katwa et al., 2012). In addition, mast cells have been shown to be involved in cerium oxide-induced alterations in vascular reactivity (Wingard et al., 2011). Even though mast cells appear to be central in the pathogenesis following NP exposure little research has been done assessing the direct interaction of NPs with mast cells. While mast cells are well-known to be involved in allergic conditions, it is currently unclear if NPs have the capacity to induce and/or promote an allergic disease state (Podila and Brown, 2013; Shannahan and Brown, 2014; Shannahan et al., 2012). One study reported that AgNPs can induce mast degranulation in the RBL-2H3 rat basophilic cell line, however, the study was focused on real-time live cell imaging of the degranulation process but not the influence of physicochemical properties of AgNPs (Yang et al., 2010). In another study, researchers used mouse peritoneal mast cells to compare uptake of spherical Au and Ag NPs (Marquis et al., 2011). This study demonstrated that positively charged NPs were internalized more than negatively charged NPs while mast degranulation was decreased in cells exposed to negatively charged AgNPs.

Scavenger receptors are well known for their role in recognizing and binding lipid molecules such as low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (Goldstein et al., 1979; Krieger and Herz, 1994; Landschulz et al., 1996). Scavenger receptor B1 (SR-B1) is a multi-ligand receptor that preferentially binds lipid molecules and other negatively charged molecules (Krieger and Herz, 1994; Landschulz et al., 1996; Rigotti et al., 1997b). Furthermore, SR-B1 has been reported to recognize and bind with pathogens and NPs (Eyre et al., 2010; Mooberry et al., 2010). Many different types of cells express SR-B1 including epithelial cells, endothelial cells, and macrophages. Specifically, the cellular uptake of AgNPs by macrophages and subsequent apoptosis has been shown to be scavenger receptor dependent (Singh and Ramarao, 2012). Therefore it is likely that other cells, which express scavenger receptors on their surface such as mast cells, may interact with AgNPs similarly and this receptor interaction may mediate toxic responses.

In this study, we hypothesized that NP physicochemical properties such as size, shape, and surface coating will influence mast cell degranulation through interaction with SR-B1. To address this hypothesis, bone marrow derived mast cells were used to assess AgNP directed degranulation using AgNPs of differing size, shape and surface coating. Lastly, we evaluated the role of SR-B1 in the observed mast cell degranulation response to various AgNPs.

2. Materials and methods

2.1. Silver nanoparticles

20 and 110 nm spherical AgNPs either suspended in citrate (C20 and C110) or polyvinylpyrrolidone (PVP) (P20 and P110) were procured through the National Centers for Nanotechnology Health Implications (NCNHIR) and initially characterized by the National Characterization Laboratory at the National Cancer Institute. Two types of nanoplates with optical resonance peak at specific wavelengths of 550 nm and 850 nm suspended in PVP (P550 and P850), or Ag nanowires that are up to 2 μm suspended in PVP were purchased from NanoComposix at a concentration of 1 mg/ml. All AgNPs were negative for endotoxin contamination.

2.2. Silver nanoparticle characterization

The hydrodynamic size and Zeta potentials (ZetaSizer Nano, Malvern) of all AgNPs were characterized in N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES). All measurements were performed with 3 individuals samples at a concentration of 50 μg/ml. The size and shape of the AgNPs were confirmed via transmission electron microscopy (TEM, Hitach 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 (the greatest distance between two points on an objects boundary).

2.3. Reagents and antibodies

SR-B1 inhibitor 2-(2-butoxyethyl)-1-cyclopentanone thosemicarbazone (Blt2) (Chembridge Corp., San Diego, CA, USA), Rat Lysosome-associated membrane proteins 2 (Lamp2) anti-mouse antibody (eBioscience Inc., San Diego, CA, USA), Imatinib ($\geqslant$98%) (Cayman Chemical Company, Ann Arbor, MI, USA)

2.4. Cell culture

Bone marrow-derived mast cells were cultured from femoral marrow cells of C57Bl/6 mice (Jackson Laboratories, Bar Harbor, ME). Bone marrow from 2 mice were used for each batch of mast cells. Cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μg/ml Primocin™ (Invivogen, San Diego, CA), 25 mM HEPES, 1.0 mM sodium pyruvate, nonessential amino acids (BioSource International, Camarillo, CA), 0.0035% 2-ME and 300 ng/ml recombinant mouse IL-3 (PeproTech, Rocky Hill, NJ). Mast cells were used following 4–6 weeks of culture at 37 °C and 5% CO_2. All animal procedures were conducted in accordance with the National Institutes of Health guidelines and approved by the University of Colorado Denver Institutional Animal Care and Use Committee. All animals were treated humanely and with regard for alleviation of suffering. Cytotoxicity of AgNPs at concentrations used in this study were evaluated by MTS and LDH assays (Promega, Madison WI) and did not induce cytotoxicity compared to the control group (data not shown). Further these concentrations were based on the evaluation of other nanoparticles which have utilized similar concentrations as performed by the NIEHS Nano GO Consortium (Xia et al., 2013).

2.5. Enhanced darkfield imaging

3×10^5 cells were exposed to AgNPs at concentration of 50 μg/ml for 1 h then washed and spun on a glass slide using a Cytospin IV (Shandon Scientific Ltd., Cheshire, UK) at 300 rpm for 5 min. Cells were then fixed in 2% paraformaldehyde solution for 10 min at 37 °C then washed with phosphate-buffered saline (PBS) three times and mounted with DAPI staining ProLong® (Life Technologies, Carlsbad, CA). Cells were then qualitatively assessed by