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Oral subchronic exposure to silver nanoparticles in rats

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ABSTRACT

Because of their extremely small size, silver nanoparticles (AgNPs) show unique physical and chemical properties, with specific biological effects, which make them particularly attractive for being used in a number of consumer applications. However, these properties also influence the potential toxicity of AgNPs. In this study, we assessed the potential toxic effects of an *in vivo* oral sub-chronic exposure to polyvinyl pyrrolidone coated AgNPs (PVP-AgNPs) in adult male rats. We also assessed if oral PVP-AgNPs exposure could alter the levels of various metals (Fe, Mg, Zn and Cu) in tissues. Rats were orally given 0, 50, 100 and 200 mg/kg/day of PVP-AgNPs. Silver (Ag) accumulation in tissues, Ag excretion, biochemical and hematological parameters, metal levels, as well as histopathological changes and subcellular distribution following PVP-AgNPs exposure, were also investigated. After 90 days of treatment, AgNPs were found within hepatic and ileum cells. The major tissue concentration of Ag was found in ileum of treated animals. However, all tissues of PVP-AgNPs-exposed animals showed increased levels of Ag in comparison with those of rats in the control group. No harmful effects in liver and kidney, as well as in biochemical markers were noted at any treatment dose. In addition, no hematological or histopathological changes were found in treated animals. However, significant differences in Cu and Zn levels were found in thymus and brain of PVP-AgNPs-treated rats.

1. Introduction

Recently, there has been an increased interest in nanoscience and nanotechnology (Seal and Karn, 2014; Formoso et al., 2015; Rai et al., 2015). Nanoparticles (NPs) are particles of very small size (1–100 nm) that consist of a core made of different materials such as metals, organic polymers or carbon, which can be covered with a coat (inorganic or organic molecules) to stabilize them in the environment (Christian et al., 2008). NPs can possess physical and chemical properties different from those with larger size making them desirable in materials science and biology. Nowadays, nanomaterials have a number of applications in the daily life (BSi Report, 2007; De Jong and Borm, 2008; Salata, 2004; Susan et al., 2009). Because of their extremely small size, NPs may have the ability to enter, translocate within, and damage living organisms. As result, an improved understanding of the potential risks and hazard assessments associated with exposure to nanomaterials is clearly necessary (Gwinn and Vallyathan, 2006; Lubick, 2008; Saiyed et al., 2011).

Silver nanoparticles (AgNPs) are among the most used nanoparticles in industry. It has been shown that AgNPs have the ability to release silver ions (Ag^{+}) in suspension partly because of its surface charge, particle size, or coating (Chernousova and Epple, 2013; Hadrup and Lam, 2014). Some studies have evaluated if the effects of AgNPs are a direct result of the NPs themselves, or rather due to the interaction with the released Ag ions (Lubick, 2008; Susan et al., 2009; Liu and Hurt, 2010; Behra et al., 2013). Ions released from AgNPs are believed to be the responsible for its antibacterial properties (Lansdown and Williams, 2007; Kumar et al., 2015). The strong antibacterial activity of these NPs makes them suitable for their use in a number of consumer products such as cosmetics, contraceptives, deodorants, and food products (Chen and Schluesener, 2008; Prabhu and Poulose, 2012; Brennan et al.,

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2015; Franci et al., 2015).

It has been also shown that exposure to AgNPs can lead to a variety of toxicological effects (Ahamed et al., 2010; EPA, 2010; Johnston et al., 2010). The special properties of NPs, which make them suitable for medical and consumer uses, are also responsible for their toxicity. Most of the current studies have been focused on *in vitro* models, suggesting that AgNPs possess cytotoxic effects providing inflammation, increased radical oxygen species (ROS) generation, mitochondrial dysfunction, DNA damage, and induction of apoptosis/necrosis cell death (AshaRani et al., 2009; Lima et al., 2012; Awasthi et al., 2013; Zhang et al., 2014).

Only a few *in vivo* studies have been performed. Most of them are focused on assessing the tissue distribution of different sizes of AgNPs after inhalation or intravenous exposure (Dziendzikowska et al., 2012). There are only a few studies through oral exposure (Loeschner et al., 2011; Van der Zande et al., 2012). Recently, some studies have investigated the neurotoxicological effects of AgNPs in brain. Apoptosis and neuronal degeneration after treatment at low doses of AgNPs, have been reported (Bagheri-Abassi et al., 2015; Xu et al., 2015). In turn, other studies showed that, depending on the route of administration, a different Ag concentration pattern in organs can be found (Susan et al., 2009). Following oral exposure, AgNPs can be absorbed across the gastrointestinal (GI) barrier, enter into the systemic circulation, and accumulate into different tissues. AgNPs have been found in kidney, liver, spleen, lung, brain and small intestine (EPA, 2010; Hadrup and Lam, 2014).

The current study was aimed at investigating tissue distribution, accumulation and excretion of PVP-AgNPs (20–30 nm) after oral subchronic administration during 90 days. Subcellular localization of PVP-AgNPs, biochemical, pathological and primary indicators of possible immune toxicity were also investigated. Moreover, we studied for the very first time, the effects of PVP-AgNPs accumulation on various metal (Fe, Mg, Zn and Cu) concentrations in different tissues.

2. Material and methods

2.1. Nanoparticles preparation

Polyvinyl pyrrolidone coated PVP-AgNPs (0.2 wt % PVP; Sky-Spring Nanomaterials, Inc., Houston, USA), with an average size 20–30 nm, were obtained as dry powder (Ag, 99.95%, PVP coated). PVP-AgNPs were resuspended in 0.9% saline and administered at concentrations of 0, 50, 100 or 200 mg AgNPs/kg/day. The main criteria followed for the selection of these doses was based on previous studies by Kim et al. (2010). PVP-AgNPs were dispersed by sonication on ice during 30 min at 35–40 W. The nanoparticles solutions were freshly prepared every day just before treatment.

2.2. Animals

Adult male Sprague Dawley rats (262 ± 17.70 g) were purchased from Charles River (Sant Germain-L'Arbresle, France). Animals were housed in a room equipped with automatic light cycles (12-h light/dark) and maintained at 22 ± 2 °C and 40%–60% humidity. Food (Panlab rodent chow, Barcelona, Spain) and tap water were offered *ad libitum* throughout the study. The experiment was approved by the Ethics Committee of Animals Research, "Rovira i Virgili" University (Tarragona, Spain).

2.3. Experimental design

After 10 acclimatization days, rats were weighed and randomly divided in four different groups (n = 12 per group). Each experimental group received 0, 50, 100 or 200 mg/kg/day of PVP-AgNPs. To evaluate the toxicological effects of PVP-AgNPs, animals were daily treated by gavage during 90 days (13 weeks), at a dose-volume of 4 mL/kg body weight either with vehicle (0.9% saline), or with the specific dose of PVP-AgNP, respectively. During the study period, the clinical signs and mortality of the rats were daily observed, while body weights and food intake were weekly recorded. For metal analysis, the week just before ending of the treatment, feces and urine of the animals were collected in individual metabolic cages. At the end of the experimental period, animals were weighed and anesthetized by an intraperitoneal injection of 75 mg/kg ketamine and 0.5 mg/kg medetomidine. Eight animals per group were used for hematological, biochemical assessment, and metal analysis. Blood was collected via the posterior cava, while liver, kidney, spleen, thymus, brain and small intestine were aseptically excised, weighed and stored at −20 °C for metal analyses. Four animals per group were used for histopathology and tissue specimen preparation for transmission electron microscope (TEM) evaluation.

2.4. Characterization of the nanoparticle suspension

The morphological characteristics of the PVP-AgNPs were analyzed by a JEOL JEM-1011 (JEOL, Tokyo, Japan) transmission electron microscope (TEM), operating at an acceleration range of voltages of 100–800 kV. The morphology of the PVP-AgNPs were analyzed by a carbon film-coated Cu grids in contact with a droplet of 4 mg/mL of PVP-AgNPs resuspended in 0.5% aqueous carboxymethylcellulose (Sigma Aldrich, San Louis, MO, USA). To reduce the risk of possible artifacts formation, all samples for TEM evaluation were prepared and analyzed on the same day in which the grids were prepared. The size of 200 particles was analyzed with a particle analysis tool to establish size distributions using the ImageJ software (Version 1.48).

2.5. Determination of the concentrations of Ag and other metals

Samples of liver, kidney, spleen, thymus, brain, ileum and urine were weighed/measured (0.5–1 g tissue or 300 μl urine) in a microsampling quartz, being 65% nitric acid (Suprapur, E. Merck) added to digest the samples. For feces, 0.5–1 g plus 0.25 ml of nitric acid 65% and 0.25 ml of hydrofluoric acid were added for digestion. The microsampling inserts were then introduced in Teflon vessels and put into a microwave oven Star D (Milestone, Sorisole, Italy) (Gómez et al., 2008). All materials were previously washed with 10% nitric acid in order to avoid any possible sample contamination. For quality control, NIST Standard Reference Material (Bovine liver 1577b; NIST, Gaithersburg, MD) was also measured in each assay. Ag, Cu and Zn concentrations were determinated by means of a computer-controlled sequential inductively coupled plasma spectrometer (ICP-MS, PerkinElmer Elan 6000), and Fe and Mg by means of a inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer Optima 8300). The recovery percentage of the reference material was higher than 93%. Detection limits were the following: 0.05 μg/kg for Ag, 0.010 μg/g for Fe and 0.10 μg/kg for Mg, 0.05 μg/g for Zn and 0.10 μg/kg for Cu.

2.6. Tissue specimen preparation for TEM evaluation

Ileum and liver of AgNPs-treated and control rats (n = 4) were aseptically excised, cut into ≈ 1 mm^3 pieces and fixed in 2% of glutaraldehyde solution in 0.1 M phosphate buffer at pH 7.0 for 24 h. Samples were washed twice with 0.1 M phosphate buffer being post-fixed with osmium tetroxide 1% solution in 0.1 M phosphate buffer for 2 h. Fixed samples were washed twice with 0.1 M phosphate buffer and dehydrated in a gradient series of