Toxicology Letters 241 (2016) 184–192

Contents lists available at ScienceDirect

Toxicology Letters

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

Toxicity of nanosilver in intragastric studies: Biodistribution and metabolic effects

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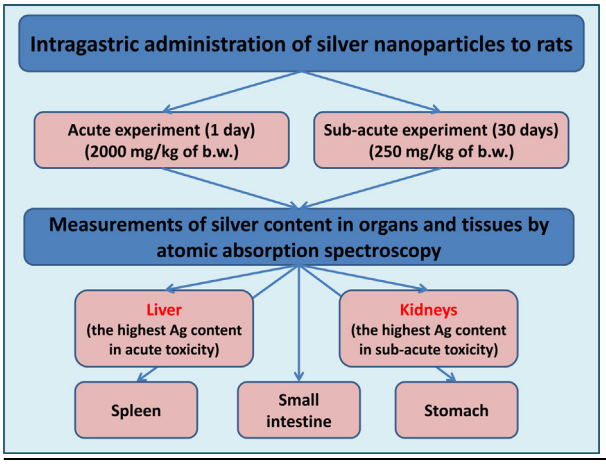
HIGHLIGHTS

- Acute and sub-acute intragastric administrations of AgNPs do not result in rats' lethality or pronounced toxic effects.
- Hematological and biochemical parameters do not change after rats' exposure to AgNPs.
- Silver absorbs from the gastrointestinal tract and distributes to various secondary organs.
- The liver and kidneys are the major target organs for AgNPs.
- Silver is efficiently excreted from rats.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form 18 November 2015
Accepted 20 November 2015
Available online 23 November 2015

Keywords:
Silver nanoparticles
In vivo toxicology
Intragastric administration
Biodistribution

ABSTRACT

The unique physicochemical properties of silver nanoparticles explain their extensive application in consumer goods, food, and medicinal products. However, the biological effects of nanosilver after peroral exposure of mammals are still debatable. This study describes the biodistribution and biological action of 12 nm non-coated silver nanoparticles intragastrically administered to male rats after acute (single exposure) and sub-acute (multiple exposures over 30 days) toxicity experiments. The daily doses were 2000 and 250 mg/kg of body weight for single and multiple administrations, respectively. Silver tissue detection was conducted by elemental analysis with the help of atomic absorption spectroscopy. An estimation of the state of exposed animals was made and the dynamics of hematological and biochemical parameters of rats was studied. It was demonstrated that single and multiple administrations resulted in silver accumulation in the liver, kidneys, spleen, stomach, and small intestine. After both one- and repeated-dose exposures, the highest Ag contents were detected in the liver (0.87 ± 0.37 μg/g of organ) and kidneys (0.24 ± 0.02 μg/g of organ). The concentrations of silver detected in tissues were far smaller

Abbreviations: AAS, atomic absorption spectroscopy; AgNPs, silver nanoparticles; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GIT, gastrointestinal tract; HPLC, high performance liquid chromatography; ICP-MS, inductively coupled plasma mass spectrometry; PBS, 50 mM potassium phosphate buffer, pH 7.4, containing 0.1 M NaCl; ROS, radical oxygen species.

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http://dx.doi.org/10.1016/j.toxlet.2015.11.018

than the administered doses (<99%), indicating its efficient excretion from the organism. Acute and subacute exposures caused no animal mortality or signs of toxicity, manifested as changes in outward appearance or notable deviations in behavior or locomotor activity. Postmortem study revealed no visible pathomorphological abnormalities of internal organs. Hematological indices and biochemical parameters of the treated rats did not differ from those of the vehicle control animals. Overall, it can be concluded that nanosilver is able to be absorbed from the gastrointestinal tract into the bloodstream and accumulate in the secondary organs of rats. It showed no distinct toxicity under the experimental conditions of this study.

1. Introduction

Nanosilver is nowadays one of the most widely known nanomaterials, with a number of production and practical applications. In ionic and colloidal form, silver has been utilized since the nineteenth century mainly as an antimicrobial agent for medical treatment (Chen and Schluesener, 2008; Ahamed et al., 2010; Prabhu and Poulose, 2012; Wei et al., 2015). Today, silver nanoparticles (AgNPs) are used in a huge number of consumer goods, including textiles, kitchenware, food storage bags and containers, clothes, sanitary and cosmetic products, baby nursing bottles, toys, and many others (Seltenrich, 2013; Yang and Westerhoff, 2014; Gaillet and Rouanet, 2015). As a food additive, nanosilver is used as an anti-caking agent, as well as to clarify beverages (Gaillet and Rouanet, 2015). Nanosilver's antibacterial properties also make it suitable for use in water purifying systems, bedding, paints, surface coatings in washing machines, and refrigerators (You et al., 2012). Due to their well-known bacteriostatic, antiviral, and antifungal action, AgNPs are extensively used as dietary supplements, for wound dressings and dental hygiene, and in implants and other medical devices (Mijnendonckx et al., 2013). AgNP applications in the treatment of breast cancer, leukemia, and different carcinomas have been proposed in several investigations (Wei et al., 2015). Beside this, AgNPs are involved in industrial processes as catalysts, and are exploited in electronics and optics (Wijnhoven et al., 2009; Zeng et al., 2010).

In a number of investigations, an appreciable release of nanosilver from functionalized materials – including clothes, textiles, and paints – into the environment has been demonstrated (Reidy et al., 2013; Yang and Westerhoff, 2014). Close day-to-day contact with goods containing nanosilver, as well as regular consumption of food products and medications containing AgNPs, is causing significant concern about the potential adverse effects of human exposure to nanoparticulate silver. The mechanism of toxicity induced by nanoparticulate silver is still unresolved, but is believed to be related to the generation of radical oxygen species (ROS) and oxidative stress resulting in apoptosis, lipid peroxidation, DNA and protein damage, membrane leakage, and other dysfunctions (Volker et al., 2013; McShan et al., 2013).

The main problem in the assessment of silver biological activity is to distinguish the effects promoted by silver in nanoparticulate form from those of dissolved Ag^+ ions released from nanoparticle surface in aqueous media. For this reason, many *in vivo* toxicological studies include simultaneous experiments on administration of soluble silver salts ($AgNO_3$, CH_3COOAg, etc.). The adverse effects of silver nanoparticles arise due to their large surface area and high reactivity. Ionic silver that is also highly reactive can diffuse across biological barriers and penetrate into cells to achieve equilibrium concentrations (Reidy et al., 2013), whereas nanoparticles absorbed through endocytosis accumulate in cells, followed by possible ion release and subsequent destructive action (this effect is known as the "Trojan horse"). Additionally, changes that can occur in the digestive tract – aggregation, interaction with biological environment molecules, or enzyme transformation – and affect the physicochemical characteristics of nanomaterial and, consequently, its bioavailability should be taken into consideration (Bohmert et al., 2014; Gaillet and Rouanet, 2015). However, the biological response to both silver forms was shown to be quite similar in many studies (Ahamed et al., 2015).

Toxic effects of ingested nano-dispersed silver and the underlying mechanisms of these effects, as well as the biodistribution of silver nanoparticles through organs and tissues, are discussed in a number of studies and reviews (Johnston et al., 2010; Reidy et al., 2013; Hadrup and Lam, 2014; Gaillet and Rouanet, 2015). A dose-dependent bioaccumulation of silver nanoparticles in different organs was demonstrated in studies of Kim et al. (2008) and Kim et al. (2010). A comparison of toxic effects and biodistribution of silver in nano- and microform was carried out by Park et al. (2010). Tissue clearance of silver nanoparticles was investigated by Lee et al. (2013). Lethality and general toxicity signs were estimated in studies of Maneewattanapinyo et al. (2011) and Kim et al. (2013).

Yu et al. (2014), studied the effects of silver nanoparticles on pregnant dams and embryo-fetal development in rats. After peroral exposure to AgNPs (~6 nm) at concentrations of 100, 300, and 1000 mg/kg/day, animals were examined for teratogenic and embryotoxic effects. No treatment-related maternal or fetal lethality or toxicity signs were observed in the exposed animals. However, alterations in glutathione reductase and catalase activities and a decrease in glutathione content in maternal liver tissues after exposure to AgNPs indicated oxidative stress. The effects of nanosilver on oxidative stress and inflammation were also investigated by Ebabe Elle et al. (2013). Rats that perorally received silver nanoparticles at a daily dose of 500 mg/kg of body weight for 81 days displayed an increase in the production of liver and cardiac oxygen radicals (30% and 41%, respectively) and raised levels of inflammatory cytokines. Exposure to AgNPs resulted in liver damage and abnormalities in lipid metabolism, with the liver and heart being the most sensitive organs to the revealed effects.

Lee et al. (2012), studied the translocation of silver nanoparticles (~8 nm with 250 mg/kg dosing) to offspring after subchronic oral exposure of pregnant dams. Silver content was found in the brain, lungs, liver, and kidneys of pups by inductively coupled plasma mass spectrometry (ICP-MS) and electron microscopy on the 4th day after parturition. High accumulation of AgNPs in the tissues of the pups was demonstrated confirming that AgNPs can overcome the blood-placental barrier.

Besides studies of nanosilver toxicity on animal models, there is a toxicological experiment on human volunteers in the literature. Munger et al. (2014) studied *in vivo* human biodistribution, bioprocessing, and toxicity of AgNPs (~5–10 and ~33 nm) at a daily dose of 100 and 480 μg/day, respectively. Fourteen-day oral dosing of nanoparticulate colloidal silver caused no evident metabolic or hematologic changes, and no alterations in urine profile, physical state, or imaging morphology. No clinically important toxicity markers were detected, and no significant changes in pulmonary reactive oxygen species or pro-inflammatory cytokine generation