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Comparative toxicity of silver nanoparticles and silver ions to *Escherichia coli*

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ABSTRACT

With the increase in silver (Ag)-based products in our lives, it is essential to test the potential toxicity of silver nanoparticles (AgNPs) and silver ions (Ag ions) on living organisms under various conditions. Here, we investigated the toxicity of AgNPs with Ag ions to *Escherichia coli* K-12 strain under various conditions. We observed that both AgNPs and Ag ions display antibacterial activities, and that Ag ions had higher toxicity to *E. coli* K-12 strain than AgNPs under the same concentrations. To understand the toxicity of AgNPs at a cellular level, reactive oxygen species (ROS) enzymes were detected for use as antioxidant enzymatic biomarkers. We have also studied the toxicity of AgNPs and Ag ions under various coexistence conditions including: fixed total concentration, with a varied the ratio of AgNPs to Ag ions; fixed the AgNP concentration and then increased the Ag ion concentration; fixed Ag ion concentration and then increasing the AgNP concentration. Exposure to AgNPs and Ag ions clearly had synergistic toxicity; however, decreased toxicity (for a fixed AgNP concentration of 5 mg/L, after increasing the Ag ion concentration) to *E. coli* K-12 strain. AgNPs and Ag ions in the presence of L-cysteine accelerated the bacterial cell growth rate, thereby reducing the bioavailability of Ag ions released from AgNPs under the single and coexistence conditions. Further works needed to consider this potential for AgNP and Ag ion toxicity across a range of environmental conditions.

Environmental significance statement: As silver nanoparticles (AgNPs)-based products are being broadly used in commercial industries, an ecotoxicological understanding of the AgNPs being released into the environment should be further considered. Here, we investigate the comparative toxicity of AgNPs and silver ions (Ag ions) to *Escherichia coli* K-12 strain, a representative ecotoxicological bioreporter. This study showed that toxicities of AgNPs and Ag ions to *E. coli* K-12 strain display different relationships when existing individually or when coexisting, and in the presence of L-cysteine materials. These findings suggest that the toxicology research of nanomaterials should consider conditions when NPs coexist with and without their bioavailable ions.

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Introduction

Silver nanoparticles (AgNPs) are often used in antimicrobial and sterile applications such as cosmetics, clothing, and medicines based on their excellent antibacterial properties (Katz et al., 2015; Silver et al., 2006; Wei et al., 2015; Zhang et al., 2009). With the increasing interest and remarkable uses of AgNPs, it is becoming essential to consider the safety of such materials and the possible risks they pose to the environment (Handy et al., 2008; Nel et al., 2006). Undeniably, the toxicity of nanoparticles (NPs) to various organisms highlights a number of research issues in the field of environmental science (Kim et al., 2012; Lu et al., 2017; Y. Wang et al., 2016), with previous reports focusing on the toxicity of AgNPs to living organisms such as *Pseudomonas putida*, *Escherichia coli*, *Daphnia magna*, *Chlamydomonas reinhardtii*, *Cyprinus carpio*, and *Euglena gracilis* (Gaiser et al., 2012; Kim et al., 2016; Li et al., 2015; Matzke et al., 2014; Navarro et al., 2008b; Sondi and Salopek-Sondi, 2004; Wu et al., 2017). Based on this previous research, three major mechanisms of AgNP toxicity to microorganisms have been suggested: (1) AgNPs can directly damage cell membranes, (2) AgNPs and silver ions (Ag ions) generate reactive oxygen species (ROS), and (3) AgNPs can release Ag ions (Marambio-Jones and Hoek, 2010).

AgNPs can also attach to the surface of living cells, where they interrupt the permeability and respiration of microorganisms (Dasgupta and Ramalingam, 2016; Steven and Fiedler, 2010). In addition, AgNPs might also act as a Trojan horse (*i.e.*, pass through cell barriers then release Ag ions inside) that damage living organisms (Lubick, 2008). Consequently, there are ongoing discussions about the roles of Ag ions that are released from AgNPs and their toxic effect on microorganisms. Some researchers have suggested that the toxicity of AgNPs is due to the NPs themselves, whereas others provide evidence that Ag ions released from AgNPs also play an important function (Park et al., 2009). When AgNPs release Ag ions, antibacterial activities are initiated by the Ag ions rather than AgNPs (Yin et al., 2011).

With regard to AgNPs- and Ag ions-based products, the major mechanism of toxicity to microorganisms is related to ROS (He et al., 2012; Hsin et al., 2008). ROS are short-lived reactive oxidants that include superoxide radicals (O_2^-), hydroxyl radicals (·OH), and hydrogen peroxide (H_2O_2) (Apel and Hirt, 2004). ROS can be generated in cells, and the oxidative stress results from a cellular defense system that includes antioxidant enzymes and antioxidants (Sondi and Salopek-Sondi, 2004). Large amounts of oxidative stress can subsequently lead to various problems and damage to proteins, lipids, and DNA (Rahal et al., 2014). Antioxidant enzymes that act as ROS scavengers include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) (Pham-Huy et al., 2008). Note that glutathione is typically present as a reduced form (GSH), where GSH is converted into its oxidized form (GSSG) *via* stimulation such as oxidative stress (Aquilano et al., 2014). In previous research, it was posited that the presence of AgNPs or Ag ions can lead to the generation of ROS, which then results in strong antibacterial activity (Maurer and Meyer, 2016; Wu and Zhou, 2013). However, there has been no precise quantitative estimate of the effect of AgNPs on *E. coli* K-12 strain that was carried out. Therefore, detecting the amount of ROS scavengers is a well-known suitable method for monitoring the toxicity assessment; thus, understanding the ROS generated by AgNPs requires detecting the amount of ROS that was scavenged. Engineered NPs can be released into the environment by various routes; for example, during manufacturing, recycling, and disposal of relevant products (Navarro et al., 2008a; Nowack and Bucheli, 2007). In one analysis of the risk of releasing AgNPs into the ecosystem, it was predicted that 15% of the total Ag released into water in the European Union would be from Ag-based products (Blaser et al., 2008). Therefore, an ecotoxicological understanding of NPs in the environment should consider that AgNPs and Ag ions coexist (Nowack et al., 2012). Various other environmental conditions, affected by anions, cations, humic acids, and pH may also influence the characteristic properties of NPs (Gao et al., 2012; Levard et al., 2012). Therefore, researchers must consider and investigate the environmental fate and behavior of NPs under diverse environmental conditions (McGillicuddy et al., 2017; Ren et al., 2016). A previous report indicated that cysteine is a strong Ag ion ligand, one that proved helpful for surveying the role of Ag ions in the general toxicity of AgNPs (Navarro et al., 2008b).The thiol (–SH) group of cysteine can readily associate with Ag ions (Levard et al., 2012).

In this paper, we investigate and compare the toxicity of AgNPs and Ag ions to *E. coli* K-12 strain. *E. coli* has been well studied in-depth knowledge of its biochemistry and genetics, which makes it the most proficient prokaryote for the investigation of toxicological assays. (Robbens et al., 2010). First, to determine the toxicity of AgNPs to *E. coli* K-12 strain, transmission electron microscopy (TEM) observations are used to bioaccumulation of AgNPs and detection of ROS was carried out. Second, we test the toxicity of AgNPs and Ag individually to *E. coli* K-12 strain. Third, we consider the toxicity of coexisting AgNPs and Ag ions to *E. coli* K-12 strain. Finally, the toxicity of AgNPs and Ag ions was combined with L-cysteine to create conditions that reduce the bioavailability of Ag ions released from AgNPs.

1. Materials and methods

1.1. *Preparation and characterization of AgNPs*

AgNP suspensions were purchased from Nanoleader, Korea and were dispersed ultrasonically (Powersonic 510, Hwasin Technology Company, Korea), and the physico-chemical properties of AgNPs were characterized. The core sizes and morphologies of the AgNPs were observed using TEM (JEOL 2100, Japan) at 200 kV and the hydrodynamic size and zeta potential were determined using dynamic light scattering (DLS; Zetasizer nano, Malvern, UK). The surface plasmon resonance was measured using a UV–Vis spectrophotometer (UV-1601PC, Shimadzu, Japan). The mass concentration of AgNPs and Ag ions in the suspension was analyzed using ultracentrifugation (Amicon 3 kDa, Millipore, 2000 ×*g*, 30 min) and HNO_3 digestion, followed by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500ce, USA) analysis; for the Ag ion solution, an $AgNO_3$ (Sigma-Aldrich, USA, >99%) stock solution was prepared in distilled water.

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