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The effect of silver nanoparticles on MCF7 breast cancer cells

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Abstract

Currently, little is known about the mechanism(s) of AgNP-induced toxicity. Many previous studies, however, have provided strong evidence for a link between the AgNP-mediated production of ROS and the subsequent generation of oxidative stress. In the current study, the effects of Ag nanoparticles on the MCF-7 breast cell line were examined, and the biomarkers related to stress oxidative including GSH, superoxide dismutase, catalase, and ROS generation were evaluated. The results showed that Ag nanoparticles induced intracellular ROS generation in a dose- and time-dependent manner. Therefore, various studies should be performed to investigate the toxic effects of this substance on different cells.

INTRODUCTION

The Ag+ ion is generally recognized as a bioactive species, and colloidal Ag has been consumed for decades owing to its perceived health benefits as an antimicrobial substance (1). The antimicrobial effect of the Ag+ ion is caused by several biological events, such as the generation of reactive oxygen species (ROS) in bacteria and the de-activation of microbial enzymes. Recently, Ag has been employed in medical and commercial uses in the form of nanoparticles (NPs) (2), which have a large surface area per unit mass, high chemical reactivity, high internal pore volumes, surface property effects, and enhanced cell penetrability as compared with other delivery systems (3). The use of NP-based consumer products is growing rapidly, and many of these products are now available in the marketplace. AgNPs are among the most commonly used nanomaterials, which is a consequence of the above-noted antimicrobial properties of Ag. AgNPs are more toxic than NPs composed of more innocuous materials, such as titanium or molybdenum (4-5). The risk of toxicity is accentuated if the AgNPs are endocytosed and deliver a bundle of Ag+ ions into the interior of cells (6). Once inside the cell, AgNPs can release the ions in the proximity of cellular organelles. Recent studies have demonstrated that common causes of AgNP-induced toxicity include oxidative stress, DNA damage, and apoptosis (7).

Environmental toxicants induce oxidative stress and alterations in the cellular redox balance (8). Oxidative stress, in turn, plays an important role in many types of cellular injury, some of which can result in DNA damage and apoptotic cell death (9). Eukaryotic organisms have evolved a comprehensive range of proteins to detoxify ROS and repair oxidative damage to DNA, lipids, and proteins. These antioxidants include enzymatic scavengers such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione S-transferase (GST), and the peroxiredoxins as well as non-enzymatic factors such as glutathione (GSH) and vitamins (10). Many studies have shown that silver nanoparticles have made their way into therapeutic applications as anti-cancer agents. Silver nanoparticles can enter the mitochondria and produce reactive oxygen species (ROS) by affecting the respiration of cells. In summary, the toxicity mechanisms of AgNPs can lead to DNA damage, oxidative stress, induction of apoptosis, and mitochondrial damage to cancer cells. In the current study, the effects of Ag nanoparticles on MCF-7 breast cell line were examined, and biomarkers related to oxidative stress, including GSH, superoxide dismutase, catalase, and ROS generation, were evaluated.

METHODS AND MATERIALS

Human breast cancer cells (MCF-7) were procured from the Iranian Biological Resource Center, preserved and subcultured in the laboratory, and used to determine cytotoxicity against Ag nanoparticles. Ag nanoparticles were suspended in the cell culture medium and diluted to appropriate concentrations (0, 10, 30, 60, and 120 µg/ml) for 24 and 48 h. Cells not exposed to Ag nanoparticles served as the control in each experiment. GSH levels were quantified

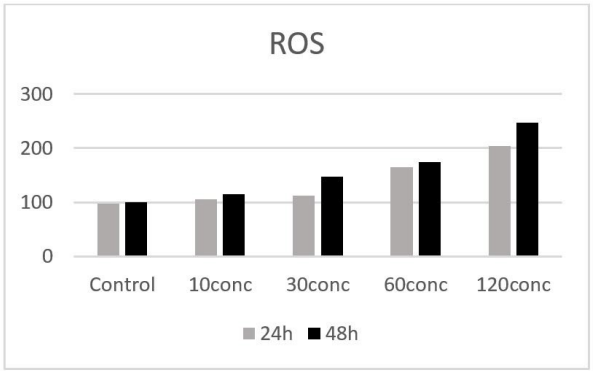
by using Ellman's reagent (DTNB). The reaction was monitored at 412 nm, and the amount of GSH was expressed in terms of nanomoles of GSH per milligram of protein. Superoxide dismutase by NTB buffer and absorbance of the reaction mixture was measured at 450 nm. Catalase activity was measured by its ability to split hydrogen peroxide (H2O2) within 1 min of incubation time. Catalase activity was expressed as micromoles of H2O2 decomposed per minute per milligram of protein. ROS generation was assessed in MCF-7 cells using DCFH-DA dye as the fluorescence agent. Data was expressed as mean (±SE) and analyzed by one-way analysis of variance (ANOVA). A *p*-value less than 0.05 was considered statistically significant.

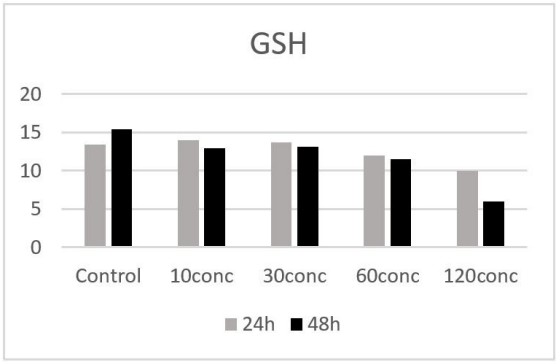
RESULTS

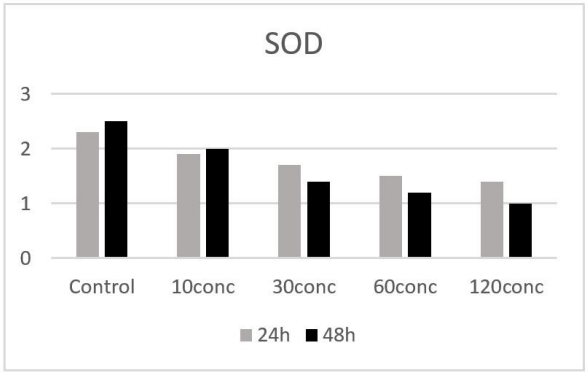
The ability of Ag nanoparticles to induce oxidative stress was measured by measuring levels of GSH, ROS, superoxide dismutase, and catalase in an MCF7 breast cell line. The results showed that Ag nanoparticles induced intracellular ROS generation in a dose- and time-dependent manner (Table 1).

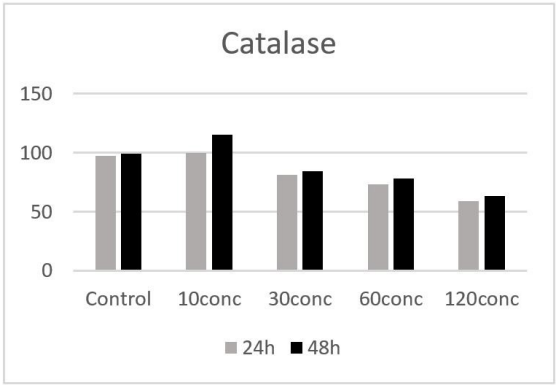
Table 1. Levels of GSH, ROS, superoxide dismutase, and catalase in a dose- and time-dependent manner

Stress oxidative biomarker	ROS (DCF-fluorescence)		GSH (nMcSH/mg)		SOD (Unit/ml)		Catalase (%of control)	
Treatment time	24h	48h	24h	48h	24h	48h	24h	48h
Control	98	100	13.4	15.4	2.3	2.5	97	99
10conc	105	115	14	12.9	1.9	2	100	115
30conc	112	147	13.7	13.1	1.7	1.4	81	84
60conc	165	174	12	11.5	1.5	1.2	73	78
120conc	204	247	10	6	1.4	1	59	63









As shown in Figs. 1a-2a-3a-4a, the amount of ROS was increased by increasing the dose and treatment time.

DISCUSSION

Many biochemical and molecular changes related to genotoxicity are initiated by AgNPs in cultured cells (11). Little is currently known about the mechanism(s) of AgNP-induced toxicity. Many previous studies, however, have provided strong evidence for a link between the AgNP-mediated production of ROS and the subsequent generation of oxidative stress (12). Recent studies have demonstrated that common causes of AgNP-induced toxicity include oxidative stress, DNA damage, and apoptosis. ROS generation, frequently detected with

a dichlorofluorescein-based probe, is induced after cells are treated with AgNPs at a concentration of 0.2 mgml–1 or higher (13). GSH is a major endogenous antioxidant scavenger that protects cells against oxidative stress through its ability to bind to and reduce ROS. Thus, preservation of the GSH-mediated antioxidant defense system is critical for cell survival (14). However, various studies have indicated that cellular levels of GSH are either increased or decreased after in vitro treatment with AgNPs. The increased levels of GSH observed in some AgNP-treated cells may involve cellular. In this study, the effects of Ag nanoparticles at concentrations of 0, 10, 30, 60, and 120 (µg/ml) for 24 and 48 h on the oxidative stress of an MCF-7 cancer cell line were evaluated (15). Oxidative stress biomarkers, ROS, GSH, SOD, and catalase were evaluated in periods of 24 and 48 hours. The results showed that Ag nanoparticles induced intracellular ROS generation in a dose- and time-dependent manner. Work with Drosophila melanogaster has likewise indicated a role for AgNPs in the stimulation of oxidative stress (16). AgNP ingestion by Drosophila activated oxidative stress pathways involving SOD, catalase, MDA, and GSH. In another study, AgNP-induced activation of SOD activity was attenuated by the administration of vitamin C, an antioxidant, to fruit fly larvae. Due to the many applications of silver nanoparticles in medicine, especially in cancer research, the toxic effects of this substance are of great importance (17). Therefore, various studies should be performed to investigate the toxic effects of this substance on different cells. It is suggested that the effects of a wide range of different antioxidants be assessed to reduce the toxic effects of the Ag-NP substance.

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