Cytotoxic Effects of Silver and Gold Nanoparticles in Human Glioblastoma U87 Cells

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ABSTRACT

Nanoparticles have contributed significantly to the design of new drug delivery and targeting in cancer chemotherapy. Consequently, some of the desirable qualities of silver and gold nanoparticles have prompted their novel applications in cancer nanobiotechnology. Glioblastoma is the most common and highest grade primary brain tumors in adults. Despite limited recent advances in treatment of this neurotumor, the prognosis of patients with this tumor remains extremely poor. In this study, we have investigated the hypothesis that silver and gold nanoparticles exert differential cytotoxic effects on human astrocytoma (glioblastoma) U87 cells. Our results demonstrate silver and gold nanoparticles induced time- and concentration-related effects in lowering the survival of U87 cells. Consistent with our hypothesis, the effects induced by silver nanoparticles were much more pronounced compared to those induced by gold nanoparticles. We also found that both silver and gold nanoparticles induced changes in the morphology of the cell body and processes of U87 cells: again, the effects of silver nanoparticles were more marked than those induced by gold nanoparticles. Our results may have pathophysiological implications in cytotoxicity of metallic nanoparticles in neural cells and suggest silver nanoparticles may have chemotherapeutic potential in the design of new treatment(s) for glioblastoma.

Keywords: Silver nanoparticles, gold nanoparticles, U87 cells, nanotoxicity, cytotoxicity of nanoparticles

1 INTRODUCTION

Because of their unique properties, many nanoparticles are being exploited in numerous applications in diverse industries [1]. Thus, it is not surprising that applications of nanoparticles in biomedical research and development have been escalating [2]. In addition to being employed as probes and in a variety of cell and tissue imaging, nanoparticles have contributed significantly to the design of new drug delivery and targeting in cancer chemotherapy [3-5].

Among metallic nanoparticles, silver and gold nanoparticles have become particularly popular in many applications because of their presumed inertness. For example, silver nanoparticles are increasingly employed in multitudes of consumer products [6] and are thus produced on a large and industrial scale [7]. Similarly, gold nanoparticles have attracted enormous scientific and technological interest owing to their ease of synthesis, chemical stability, and unique optical properties [8]. Consequently, some of the desirable qualities of these two metallic nanoparticles have prompted their novel applications in cancer nanobiotechnology.

Glioblastoma is the most common primary brain tumors in adults. It is the highest grade (grade IV as defined by the World Health Organization) astrocytoma and is characterized by aggressive and non-stoppable proliferation and invasion into the surrounding normal tissues [9, 10]. Despite some limited recent advances in treatment of this neurotumor, the prognosis of patients with this tumor remains extremely poor [9, 10]. Consequently, there is a real urgent need for new and more effective therapies for managing this devastating disease [10].

Our group has established a history of developing many neural and non-neural cell types as *in vitro* models for

systematic investigation of putative cytotoxicity of various nanomaterials, including metallic and non-metallic nanoparticles, in neural and non-neural cells [1, 11-17]. In this study, we have investigated the hypothesis that silver and gold nanoparticles exert differential cytotoxic effects on human astrocytoma (glioblastoma) U87 cells.

2 MATERIALS AND METHODS

2.1 Materials

Human astrocytoma (astrocytes-like) U87 cells were obtained from ATCC (Manassas,VA, USA). Thiazolyl blue tetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St Louis, MO, USA). Fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Lawrenceville, GA, USA). Tetrachloroauric (III) acid ($HAuCl_4 \bullet 3H_2O$), trisodium citrate ($C_6H_5Na_3O_7 \bullet 2H_2O$) and silver nitrate ($AgNO_3$) were purchased from Fisher Scientific (Pittsburgh, PA, USA). All chemicals were of analytical grade unless otherwise stated.

2.2 Cell Culture

U87 cells were cultured in an incubator at 37˚ C and 5 % (v/v) CO_2 in Minimum Essential Medium (MEM) (Sigma, St Louis, MO, USA) supplemented with 10% (v/v) FBS.

2.3 Cell viability assay

Cellular viability was determined by using the modified MTT assay [12-14]. Cells were seeded into the wells of 24-well plates at a density of 4000 cells/well in the presence of 0 (control), 1, 25, 50, 75, 125, or 250 µL silver or gold nanoparticles and cultured as described above. (Silver and gold nanoparticles were prepared as described previously [18].) At the end of the specified culture period, MTT dye (0.5% (w/v) in PBS) was added to each well and the plates were incubated for an additional 4 hours at 37°C. The purple-colored insoluble formazan crystals in viable cells were dissolved using 200 µL DMSO and the subsequent absorbance (designated as X) of the content of each well was measured at 570 nm using a Bio-Tek Synergy HT Plate Reader (Winooski, VT, USA) as described previously [14].

The silver and gold nanoparticles by themselves had absorbance: thus, their absorbance (i.e., the control sets of wells) had to be subtracted from the absorbance of live cells with different treatments as depicted in the preceding paragraph. The control sets of wells were set up alongside those sets of well in the plates as detailed in the preceding paragraph except that the control sets of wells did not contain any seeded U87 cells. At the end of the specified culture period, 100 µL of MTT dye (0.5% (w/v) in PBS) was added to each well and the plates were incubated for an additional 4 hours at 37°C. The subsequent absorbance (designated as Y) of the content of each well was measured at 570 nm as described above. (X-Y) was taken as the absorbance attributed to viable cells in each well.

2.4 Confocal laser scanning microscopy

U87 cells were cultured on coverslips in 6-well plates in the presence or absence (i.e., the control) of 75 µL of silver or gold nanoparticles for 7 days. Then the cells were washed twice with PBS and fixed with 4% (w/v) paraformaldehyde at room temperature for 15 minutes. Subsequently, the fixed cells were washed with PBS to remove the remaining paraformaldehyde and were examined and their images captured using an Olympus (Center Valley, PA, USA) FV1000 confocal laser scanning microscope.

2.5 Statistical analysis of data

Experiments were performed at least three times with a minimum of 6 replicates for each set, and all data were recorded as mean ± standard deviation (shown in figures).

3 RESULTS AND DISCUSSION

Employing the modified MTT assay, we systematically compared the effects of silver nanoparticles with gold nanoparticles on U87 cells (Figure 1 and 2). As shown in Figure 1, exposure of U87 cells to silver nanoparticles induced concentration- and time-related decreases in survival, proliferation, and/or growth of the U87 cells.

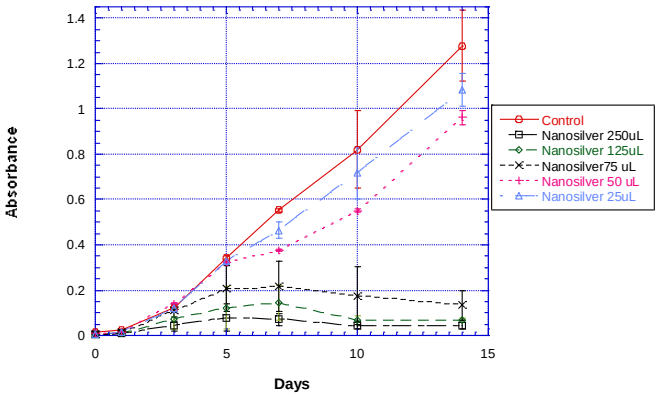

Figure 1. Effect of different concentrations of nanosilver particles on survival, proliferation and/or growth of U87 cells for 14 days.

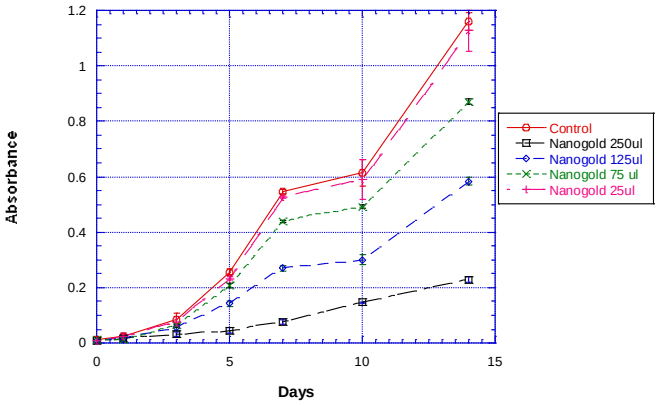

Figure 2. Effect of different concentrations of nanogold particles on survival, proliferation and/or growth of U87 cell for 14 days.

Exposure of U87 cells to gold nanoparticles (Figure 2) also induced concentration- and time-related decreases in survival, proliferation, and/or growth of the cells. However, the effects induced by silver nanoparticles were much more pronounced compared to those induced by gold nanoparticles, especially at the higher treatment concentrations (compare Figure 1 with Figure 2). These results suggest that silver nanoparticles are generally more cytotoxic to U87 cells compared to gold nanoparticles.

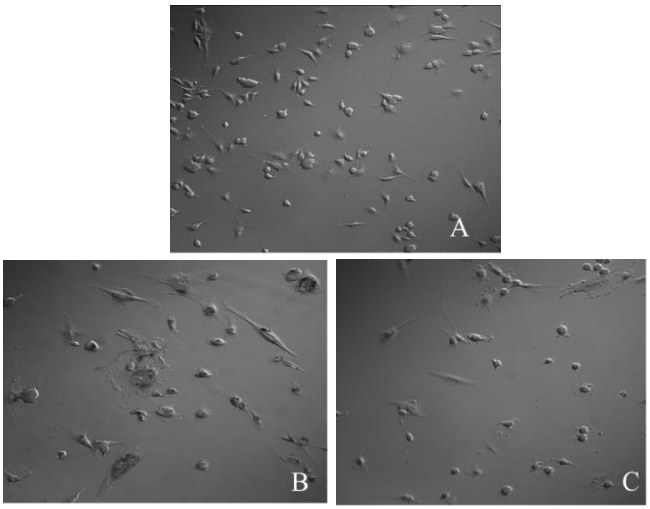

Figure 3. Effects of silver and gold nanoparticles on morphology of U87 cells. U87 cells were exposed to silver or gold nanoparticles for 7 days and then examined with confocal microscopy at a magnification of 200: A) control (i.e., untreated) U87 cells; B) cells treated with 75 µL of silver nanoparticles; and C) cells treated with 75 µL of gold nanoparticles.

We also monitored if the treatments with the two types of nanoparticles induced any changes in the morphology of U87 cells. As noted under confocal light microscopy, the control (i.e., untreated) U87 cells showed "healthy" morphology with one or more processes extended from their cell bodies (Figure 3A). After U87 cells had been treated with 75 µL of silver nanoparticles (Figure 3B) or gold nanoparticles (Figure 3C) for 7 days, fewer live cells were observed per field compared to that of the control. Furthermore, the nanoparticles-treated cells appeared to extend fewer processes from their cell bodies. At the same time, some silver nanoparticles aggregated on the U87 cells (Figure 3B).

Additionally, although some of the U87 cells treated with both nanoparticles appeared to be swollen, many more of the U87 cells treated with silver nanoparticles showed swollen cell bodies compared to either the control U87 cells or even the cells treated with gold nanoparticles (compare Figures 3A, 3B and 3C). Thus, our morphological findings are consistent with the notion that silver nanoparticles are more cytotoxic than gold nanoparticles to U87 cells. This conclusion is compatible with our results obtained using cell survival assays (Figures 1 and 2).

4 CONCLUSIONS

Our previous and ongoing studies demonstrate that silver and gold nanoparticles induced time- and concentration-related effects in lowering the survival of U87 cells. The effects induced by silver nanoparticles were much more pronounced compared to those induced by gold nanoparticles. We also found that both silver and gold nanoparticles induced changes in the morphology of the cell body and processes of U87 cells: again, the effects of silver nanoparticles were more marked than those induced by gold nanoparticles. Our results may have pathophysiological implications in cytotoxicity of metallic nanoparticles in neural cells and suggest silver nanoparticles may have chemotherapeutic potential in the design of new treatment(s) for glioblastoma [10].

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