



Effect of silver nanoparticles on cytotoxicity, oxidative stress and pro-inflammatory proteins profile in lung adenocarcinoma A549 cells

Magdalena Matysiak-Kucharek^{1,A-D}✉, Krzysztof Sawicki^{1,C,E}, Lucyna Kapka-Skrzypczak^{1,2,E-F}

¹ Department of Molecular Biology and Translational Research, Institute of Rural Health, Lublin, Poland

² World Institute for Family Health, Calisia University, Kalisz, Poland

A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

Matysiak-Kucharek M, Sawicki K, Kapka-Skrzypczak L. Effect of silver nanoparticles on cytotoxicity, oxidative stress and pro-inflammatory proteins profile in lung adenocarcinoma A549 cells. *Ann Agric Environ Med.* 2023; 30(3): 566–569. doi: 10.26444/aaem/169214

Abstract

Introduction and Objective. The general population is exposed to AgNPs released into the environment, e.g. through the respiratory tract. Lung cancers are among the most frequently diagnosed and deadly malignancies, often diagnosed at late stage with existing distant metastases. The aim of the study was to determine the activity of AgNPs against A549 lung cancer cells.

Materials and method. A549 cells and AgNPs were used in the study. Cytotoxicity was tested by MTT and NR assays. Oxidative stress was determined by measuring malonyldialdehyde and level of free -SH groups. Proteins secretion was assessed using the Human Profiler Cytokine Array Kit assay.

Results. AgNPs reduce A549 cells viability and induce oxidative stress. They also lead to increased secretion of several proinflammatory proteins, which stimulate metastasis.

Conclusions. AgNPs exhibit direct anti-cancer effect, however, their potentially promethastic effect encourages further work on the safety of nanomaterials.

Key words

lung cancer, cytotoxicity, inflammation, metastasis, silver nanoparticles, A549, oxidative stress

INTRODUCTION

Among the most numerous used nanomaterials, silver nanoparticles (AgNPs) occupy the leading position. Due to their unique antimicrobial attributes, they have been used in many fields, such as medicine, cosmetology, textiles, renewable energy or environmental protection. They are also widespread in agriculture and crop production, where they are used in the production of fertilizers and plant growth stimulants [1]. Due to the prevalence of their use, it can be said that the general population, among agricultural workers especially, is exposed to AgNPs being released into the environment. Silver nanoparticles can get into the human body primarily by inhalation, ingestion and through the skin. Nevertheless, the respiratory tract is the key to AgNPs toxicity, and respiratory system diseases are an increasing threat to human health. In turn, lung cancer is one of the most frequently occurring malignancies worldwide. The disease is characterized by a relatively late diagnosis, poor prognosis, and short survival. The diagnosis is often already made at the stage of distant metastasis to the lymph nodes, bones, liver and brain.

OBJECTIVE

The aim of the study was to determine the board-spectrum activity of AgNPs against A549 lung adenocarcinoma cells by assessing their cytotoxicity, impact on the level of oxidative stress markers, and assessment of the impact on the secretion of a number of cytokines, chemokines, acute-phase proteins and growth factors.

MATERIALS AND METHOD

Cell culture. The research was carried out on human lung adenocarcinoma cells of the A549 line, purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured as recommended by the manufacturer in Ham's F12 growth medium with 10% fetal bovine serum at the temperature of 37°C and in 5% CO₂ atmosphere.

AgNPs preparation. Stock solutions were prepared by dissolving 2 mg of 20 nm AgNPs (PlasmaChem, Berlin, Germany) in 800 µL distilled water. The solution was sonicated and 100 µL of 10× concentrated PBS and 100 µL of bovine serum albumin 15% solution added. The remaining dilutions were prepared by adding the appropriate amount of cell medium to the stock solution. The physicochemical parameters of the tested AgNPs in the used cellular medium have been described previously [2].

MTT assay. A549 cells (1×10⁴ cells/well in 100 µL of culture medium) were seeded on 96-well microplates and allowed

✉ Address for correspondence: Magdalena Matysiak-Kucharek, Department of Molecular Biology and Translational Research, Institute of Rural Health, Jaczewskiego 2, 20-090 Lublin, Poland
E-mail: matysiak.magdalena@imw.lublin.pl

Received: 05.04.2023; accepted: 04.07.2023; first published: 21.07.2023

to grow for 24h. The cells were then treated with appropriate AgNPs solutions and incubated for 24h or 48h. 15 μ l of 5 mg/mL stock solution of MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well and incubated for 3 hours at 37°C. After the fluid was poured out, 100 μ l of dimethyl sulfoxide was added to the wells and subjected to 2 minutes of vigorous shaking. Finally, the optical density was read at the wavelength of 570 nm.

NR assay. A549 cells (2×10^4 cells/well in 200 μ l of culture medium) were seeded on 96-well microplates and incubated overnight, after which the medium was replaced with AgNPs solutions. Respectively, after 24 or 48 hours, the medium was removed and cells washed with 150 μ l PBS. Then, 100 μ l of neutral red solution (50 μ g/mL) was added and incubated for 4h at 37°C. In the last step, after removing the NR solution, the cells were washed with a 150 μ l of PBS, and treated with a 200 μ l of solvent (the solution contains ethanol – 50%, water – 49% and acetic acid – 1%). NR absorbance was measured spectrophotometrically at 540 nm, after 20 minutes smooth shaking.

Supernatants and cell pellets. A549 cells were seeded on 6-well plates. After overnight incubation, cells' medium was replaced with fresh AgNPs solutions. 48 hours later, the supernatant was collected. The cells were washed with PBS, trypsinized and harvested. After centrifugation, they were washed twice with PBS. Both supernatants (-20°C) and cells (-80°C) were frozen for further analysis. Supernatants were collected to perform Proteome Profiler Human XL Cytokine Array Kit assay and cells to measure oxidative stress markers. A more detailed description of the sample preparation and assays methodology can be found in our previous manuscript [3].

Malondialdehyde (MDA) level. Commercially available QuantiChrom™ TBARS Assay Kit (BioAssay Systems, Hayward, CA, USA) was performed to measure the MDA level in AgNPs treated A549 cells.

-SH group level. Fluorometric Thiol Assay Kit (Sigma-Aldrich, Saint Louis, MO, USA) was performed to measure thiol group relative level in AgNPs treated A549 cells.

Proteome Profiler Human XL Cytokine Array Kit. Semi-quantitative Proteome Profiler Human XL Cytokine Array Kit (R&D Systems, Minneapolis, MN, USA) was prepared in accordance with the manufacturer's recommendations, to determine the protein profile of AgNPs treated A549 cells. Fiji software (NIH, Bethesda, MD, USA) was used for quantitating the signal emitted by the protein bands of interest. The average for points corresponding to one specific protein was calculated, background subtracted and the result normalized to reference points.

Statistical analysis. Statistical significance was assessed by ANOVA and parametric Tukey's test was used as a *post-hoc* analysis. The p -value ≤ 0.05 was established as statistically significant. Calculations were performed in Prism 9 (GraphPad Software, San Diego, CA, USA) and charts prepared on Microsoft Excel (Microsoft Corporation, Redmond, WA, USA).

RESULTS

Cytotoxicity. The presented study shows that the tested AgNPs affected the viability of A549 cells in a concentration- and incubation-time dependent manner. Both the effect on mitochondrial metabolism measured by the MTT (Fig. 1A) assay and the integrity of cell membranes assessed by the NR assay (Fig. 1B) were demonstrated. However, in both tests, the cytotoxicity found was relatively low and it was not possible to calculate reliable IC50 values, taking into account the fact that at the maximum tested concentration, i.e. 200 μ g/ml, cells viability was still more than 70%.

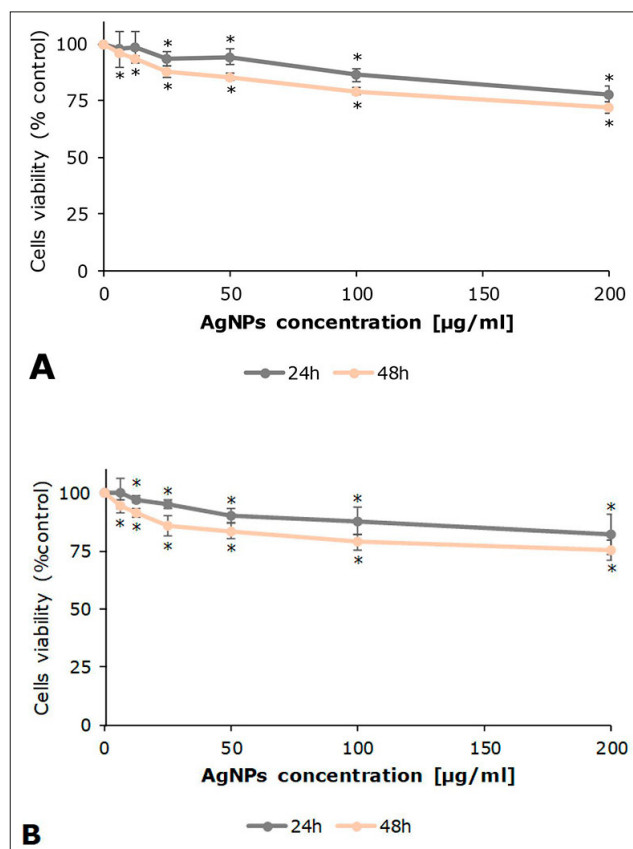


Figure 1. 20 nm AgNPs cytotoxicity against human lung adenocarcinoma A549 cells measured by MTT (A) and NR (B) assays

Oxidative stress. The next stage of the research was to determine the effect of the tested AgNPs on the level of oxidative stress in A549 cells. The level of oxidative stress was assessed using indirect methods. Tested AgNPs have been shown to significantly increase the production of MDA, the end product of lipid peroxidation (Fig. 2A). At the same time, they reduce the amount of free -SH groups, which proves the impairment of anti-oxidant mechanisms (Fig. 2B).

Inflammation. Using the semi-quantitative Proteome Profiler Human XL Cytokine Array Kit (R&D Systems, Minneapolis, MN, USA) the impact of AgNPs on the level of secretion of 105 pro-inflammatory proteins was assessed. A549 cells have been shown to secrete 12 of the proteins tested. For C-X-C motif chemokine ligand 10 (CXCL10), interleukin-1 receptor antagonist protein (IL-1ra), interleukin 1 beta (IL-1 β) and interleukin 15 (IL-15), the level of secretion was at the detection limit of the assay; therefore, no further quantification was

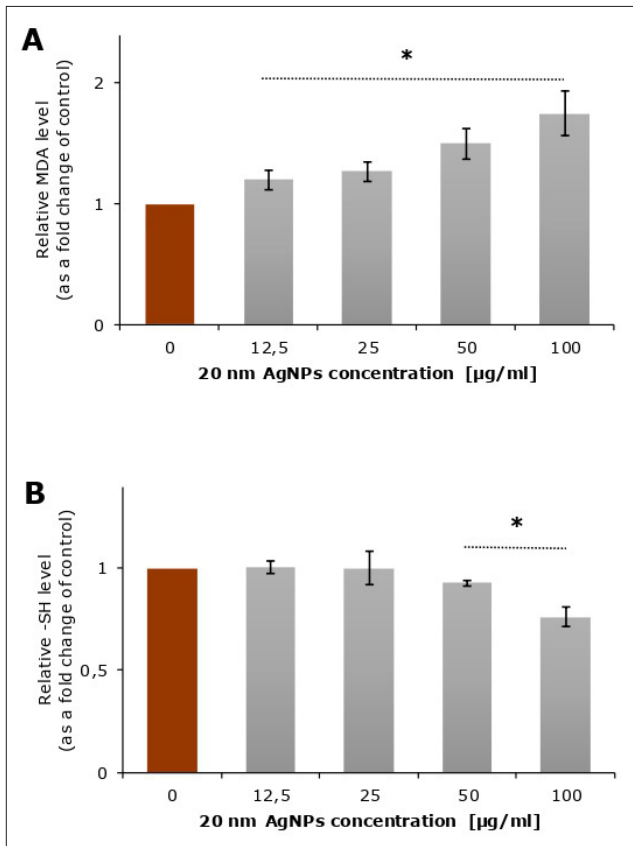


Figure 2. 20 nm AgNPs impact on oxidative stress parameters in A549 cells lysates measured by MDA (A) and free -SH groups (B) assays

performed. AgNPs significantly affected the relative level of expression of vascular endothelial growth factor (VEGF), interleukin 6 (IL-6), interleukin 8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1). For granulocyte colony-stimulating factor (G-CSF) and interleukin 17A (IL-17A), the values were similar to the untreated control. Interleukin 6, which was not present in control cells, was present in the medium after AgNPs stimulation (Fig. 3).

DISCUSSION

In vitro cytotoxicity of AgNPs is widely described in the literature [4, 5]. It is strictly dependent on a number of factors, e.g. cell line used and AgNPs physico-chemical parameters, and should be considered individually for each experimental model. The current study has shown that AgNPs reduce the viability of A549 cells, which confirms the results of other researchers. Scientific studies indicate that the main mechanism of AgNPs cytotoxicity against A549 cells is the induction of oxidative stress, which is also confirmed by the results of the current study [6, 7, 8]. Oxidative stress may be a direct cause of inflammation, which is why the effect is assessed of AgNPs on the secretion of a number of pro-inflammatory proteins by A549 cells – cytokines, chemokines, acute phase proteins and growth factors. Studies have shown that A549 cells secrete only some proteins from over 100 tested by Human Profiler Cytokine Array Kit. There are two possibilities – the remaining proteins are not secreted, or are secreted in an amount lower than the assay detection limit.

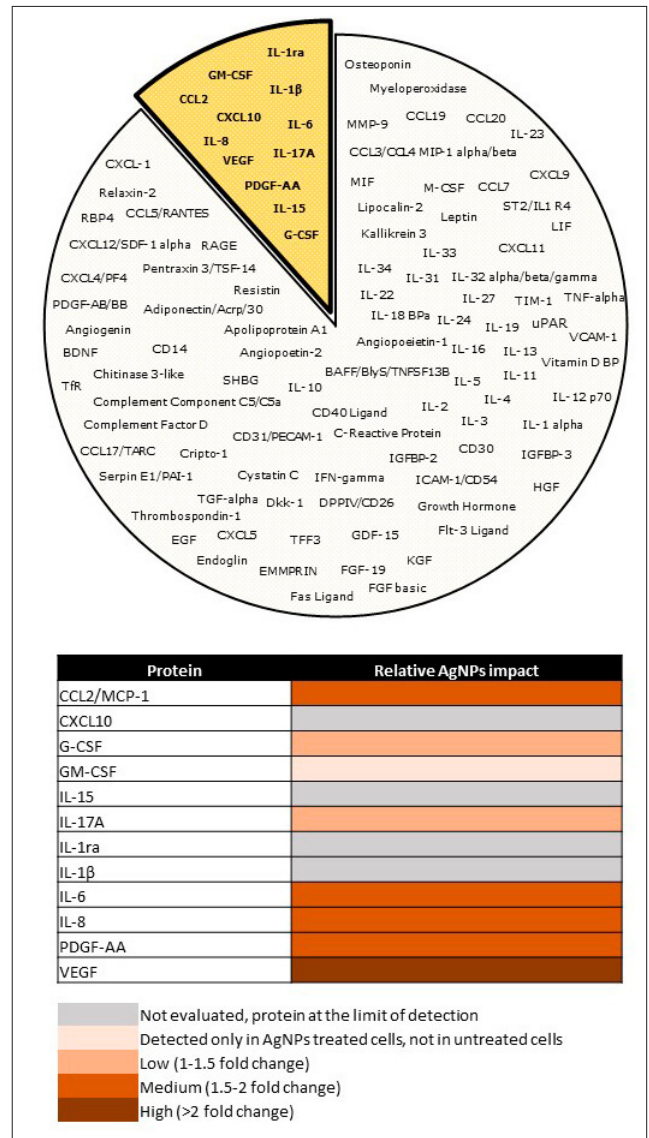


Figure 3. The effect of AgNPs on the relative level of pro-inflammatory proteins secretion by A549 cells measured by Human Profiler Cytokine Array Kit

To the best knowledge of the authors of this study, the studies described in the following manuscript are the first in the literature this is the first to assess the broad profile of pro-inflammatory proteins secreted by A549 cells treated with AgNPs. So far, research has focused mainly on the determination of single, key pro-inflammatory interleukins, such as tumour necrosis factor alpha, interleukin 6 and interleukin 8 [8, 9, 10]. The simultaneous determination of such a large number of pro-inflammatory proteins allowed for a comprehensive assessment. What seems to be crucial is the fact that the factors, the increased secretion of which is favoured by the tested AgNPs, have a proven prometastatic effect. For example, they can increase the level of migration and invasiveness of cells, promote and be involved in epithelial-mesenchymal transition, stimulate lymphangiogenesis and immune cell infiltration, and have a strong pro-angiogenic effect [11, 12].

CONCLUSIONS

The study has shown that AgNPs reduce the viability of A549 cells, induce oxidative stress and inflammation, results which indicate a direct anti-cancer effect of AgNPs. However, the proteins secreted by A549 cells are proteins that are characterized by pro-metastasis activity. This is of great concern due to the widespread use of AgNPs. Taking into account the fact that lung cancers are among the most frequently diagnosed and lethal types of cancer, the obtained results encourage further research.

Acknowledgements

The work was financed as part of the research project number 21002, financed from the resources of the Institute of Rural Health in Lublin for the maintenance and development of research potential.

REFERENCES

1. Kale SK, Parishwad GV, Husainy ASN, et al. Emerging Agriculture Applications of Silver Nanoparticles. *ES Food Agrofor*. 2021;3:17–22. <https://doi.org/10.30919/esfaf438>
2. Lankoff A, Sandberg WJ, Wegierek-Ciuk A, et al. The effect of agglomeration state of silver and titanium dioxide nanoparticles on cellular response of HepG2, A549 and THP-1 cells. *Toxicol Lett*. 2012;208(3):197–213. <https://doi.org/10.1016/j.toxlet.2011.11.006>
3. Matysiak-Kucharek M, Czajka M, Jodłowska-Jędrych B, et al. Two Sides to the Same Coin—Cytotoxicity vs. Potential Metastatic Activity of AgNPs Relative to Triple-Negative Human Breast Cancer MDA-MB-436 Cells. *Molecules*. 2020;25(10):2375. <https://doi.org/10.3390/molecules25102375>
4. Zhang N, Xiong G, Liu Z. Toxicity of metal-based nanoparticles: Challenges in the nano era. *Front Bioeng Biotechnol*. 2022;10:1001572. <https://doi.org/10.3389/fbioe.2022.1001572>
5. Garcés M, Cáceres L, Chiappetta D, et al. Current understanding of nanoparticle toxicity mechanisms and interactions with biological systems. *New J Chem*. 2021;45:14328–14344. <https://doi.org/10.1039/D1NJ01415C>
6. Gurunathan S, Kang M, Kim JH. Combination Effect of Silver Nanoparticles and Histone Deacetylases Inhibitor in Human Alveolar Basal Epithelial Cells. *Molecules*. 2018;23(8):2046. <https://doi.org/10.3390/molecules23082046>
7. Li J, Chang X, Shang M, et al. Mitophagy–lysosomal pathway is involved in silver nanoparticle-induced apoptosis in A549 cells. *Ecotoxicol Environ Saf*. 2021;208:111463. <https://doi.org/10.1016/j.ecoenv.2020.111463>
8. Shimizu K, Kashiwada S, Horie M. Cellular Effects of Silver Nanoparticle Suspensions on Lung Epithelial Cells and Macrophages. *Appl Sci*. 2022;12(7):3554. <https://doi.org/10.3390/app12073554>
9. Sun J, Wan J, Zhai X, et al. Silver nanoparticles: Correlating particle size and ionic Ag release with cytotoxicity, genotoxicity, and inflammatory responses in human cell lines. *Toxicol Ind Health*. 2021;37(4):198–209. <https://doi.org/10.1177/0748233721996561>
10. González-Vega JG, García-Ramos JC, Chavez-Santoscoy RA, Castillo-Quiñones JE, Arellano-García MA, Toledano-Magaña Y. Lung Models to Evaluate Silver Nanoparticles' Toxicity and Their Impact on Human Health. *Nanomaterials*. 2022;12(13):2316. <https://doi.org/10.3390/nano12132316>
11. Morris RM, Mortimer TO, O'Neill KL. Cytokines: Can Cancer Get the Message? *Cancers (Basel)*. 2022;14(9):2178. <https://doi.org/10.3390/cancers14092178>
12. Chattopadhyay I, Ambati R, Gundamaraju R. Exploring the Crosstalk between Inflammation and Epithelial-Mesenchymal Transition in Cancer. *Mediators Inflamm*. 2021;2021:9918379. <https://doi.org/10.1155/2021/9918379>