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Silver Nanoparticles as Nanoantibiotics: A Comparative Analysis of their Toxicity on Biological Systems of Different Complexity

Nanopartículas de plata como nanoantibióticos: Un análisis comparativo de su toxicidad en sistemas biológicos de diferente complejidad

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Resumen. - *Actualmente, las nanopartículas de plata (AgNPs) se estudian ampliamente para aplicaciones biomédicas, pero a pesar de que los nanomateriales proporcionan muchos beneficios, recientemente su toxicidad comparativa apenas se ha explorado. En el trabajo actual, la toxicidad de AgNPs en sistemas biológicos de diferentes niveles de complejidad se evaluó de forma exhaustiva y comparativa. Los organismos incluyen virus, bacterias, microalgas, hongos, células animales y humanas (incluidas líneas celulares cancerosas). Encontramos que el crecimiento de los sistemas biológicos de diferentes grupos taxonómicos -in vitro, a nivel celular- se inhibe a concentraciones de AgNP dentro del mismo orden de magnitud (101 µg / ml). Por lo tanto, la toxicidad de AgNPs no depende de la complejidad de los organismos. El hecho de que las células y los virus se inhiban con una concentración de AgNP dentro del mismo orden de magnitud podría explicarse teniendo en cuenta que la plata afecta las estructuras fundamentales de las células y virus por igual.*

Palabras clave: Nanopartículas de Plata; Nanotoxicología; Complejidad de Sistemas Biológicos; Actividad Antimicrobiana.

Abstract. - *Currently, silver nanoparticles (AgNPs) are extensively studied for biomedical applications, but although nanomaterials provide many benefits, recently their comparative toxicity have barely been explored. In the current work, AgNPs toxicity on biological systems of different levels of complexity was assessed in a comprehensive and comparatively way. The organisms included viruses, bacteria, microalgae, fungi, animal and human cells (including cancer cell lines). We found that growth of biological systems of different taxonomical groups –in vitro, at a cellular level- is inhibited at concentrations of AgNPs within the same order of magnitude (101 µg/ml). Thus, the AgNPs toxicity does not depend on the complexity of the organisms. The fact that cells and virus are inhibited with a concentration of AgNPs within the same order of magnitude could be explained considering that silver affects fundamental structures for cells and virus alike.*

Keywords: Silver Nanoparticles; Nanotoxicology; Biological Systems Complexity; Antimicrobial Activity.

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1. Introduction

Silver nanoparticles (AgNPs) are among the most studied nanomaterials due to all their applications. In the medical field, AgNPs display antimicrobial and antiviral activity [1]. Although their many benefits, their relative toxicity has not been appropriately addressed. The few published studies of toxic effects of AgNPs on biological systems, i.e. viruses, bacteria or human cells, report different and even contradictory results. The research of the AgNPs comparative toxicity on different biological systems has become a priority task.

It is generally assumed that the toxicity of AgNPs decreases as the complexity of biological systems rises [2–6]; but some studies suggest there is no such a difference [7, 8]. Still, comparative analyses of the toxic effects of a single silver nanoparticle on biological systems of different level of complexity are scarce and are not extensive [5, 9]. Also, antimicrobial tests for AgNPs are not standardized leading to limited capacity to assess the toxic effect of AgNPs on different taxa.

We performed a comparative analysis of the toxic effects of a single type of AgNPs, on biological systems of different cellular / structural complexity, from viruses to human cancer cell lines. Also, the implications of such analysis are discussed.

2. Methodology

2.1. Virus, microorganisms, cells and culture conditions

For the systematic, comparative analysis, we used the following biological models: Rift Valley Fever Virus (RVFV) MP12 strain from the National Research Institute of Agricultural and Food Technology, Spain; The bacteria *Escherichia coli* DH5α (Gram-negative) and *Staphylococcus aureus* (Gram-positive), from the Centro de Nanociencias y Nanotecnología – Universidad Nacional Autónoma de México; the microalgae *Rhodomonas* sp., from the Faculty of Marine Sciences of the Universidad Autónoma de Baja California; The fungi *Candida albicans* ATCC SC5614 (dimorphic yeast) and *Fusarium oxysporum*, Race III, (filamentous), from the Centro de Investigación Científica y Educación Superior de Ensenada; the mammalian cells were Dendritic Cells from Murine models and Vero cells (ATCC CCL-81); the human cancer cells lines HeLa and MDA-MB-231 from the ATCC.

2.2 AgNPs preparation and characterization

The AgNPs are stabilized with Polyvinylpyrrolidone (PVP). These PVP-AgNPs were obtained from Vector Vita Ltd® (Russia). AgNPs were analyzed by UV-Vis spectrophotometry (Multiskan Go, Thermo Scientific), λ= 200-800 nm. Also, an FT-IR spectroscopy analysis was performed λ=4000-400 cm^{-1} (Nicolet 6700; Thermo Scientific). The morphology was examined by Transmission Electron Microscopy in a Jeol JEM 2100. AgNPs dilutions were prepared in a range from 0.001 to 100 μg/ml of silver.

2.3 Toxicological assays

For the virus infection assays, 0.3 to $3x10^2$ plaque-forming units (PFU) of RVFV were incubated with AgNPs. After, viruses were inoculated onto Vero cells grown in MW6 plates for 1 h, washed; and then semi-solid medium with agar was added. Plates were incubated until infection plaques were clearly developed, then were fixed and stained with crystal violet dye. For bacteria and fungi, the M09 and M27 microdilution assays, respectively, from the Clinical Laboratory Standard Institute were used, with some modifications (YPD culture media for fungi). Microbial cultures were exposed to AgNPs. Inhibition was measured by UV-Vis spectrophotometry. Microalgae inoculum was adjusted to an optical density of 0.065 at λ= 670 nm in a Jenway 6505 UV-Vis spectrophotometer. Microalgae were cultured in F2 media, and exposed to AgNPs, for 24 h, at room temperature, and under continuous light conditions. Murine bone marrow derived dendritic cells were grown in RPMI culture media, supplemented with 10 % FBS, 1% of streptomycin-penicillin G, at 37 °C with 5% CO_2 atmosphere. Cytotoxicity was evaluated with the dual fluorescein diacetate /ethidium bromide test after 24 h of cell incubation with AgNPs. Vero cells were seeded in 96-multiwell plates with DMEM media, and 24 h later, the AgNPs were added to the medium, then incubated for 24 h more, at 37 ºC. Viability was evaluated by the MTS Cell Proliferation Assay (Promega). HeLa and MDA-

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MB-231 cancer cell lines were cultured in RPMI-1640 or DMEM media supplemented with 10% FBS, 1 % penicillin-streptomycin, 1 % L-glutamine and 1.5 g/l sodium bicarbonate. Cells were maintained at 37 °C and 5 % CO_2. Cell viability was assessed by the MTT method reported by Mosmann [10].

3. Results and Discussions

3.1 AgNPs characterization

AgNPs are spheroids of 35 ± 15 nm in diameter (fig. 1a). UV-Vis analysis shows a peak at 410 nm, which is typical for metallic silver nanoparticles (fig. 1b). FT-IR measurements of lyophilized AgNPs presented a profile similar to the PVP standard.

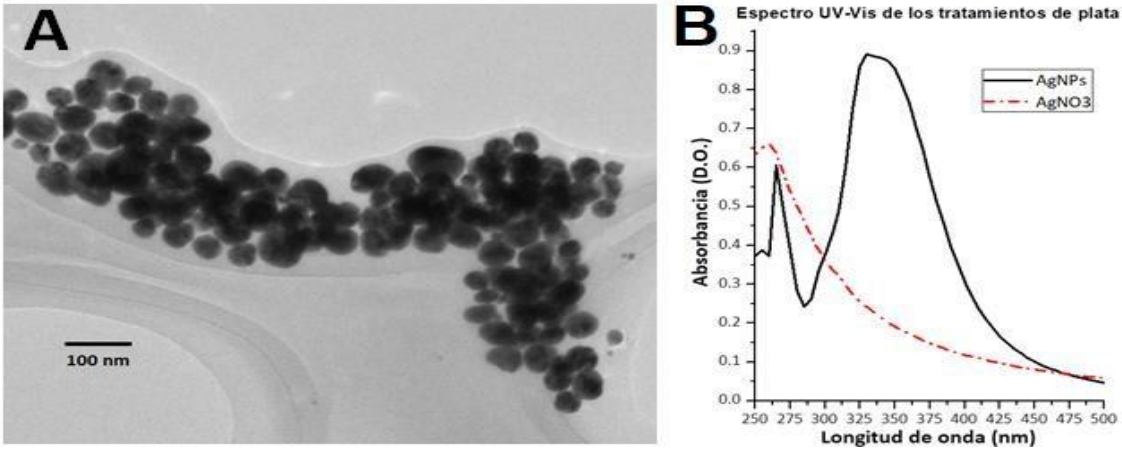

Figure 1. AgNPs characterization. TEM micrography and UV-Vis profile.

3.2 Toxicological assays

Plaque-forming units of RVFV were reduced by 98 % at a concentration of 12 µg/ml of silver. The minimum inhibitory concentration (MIC) for the bacterial strains *E. coli* and *S. aureus*, was 12 µg/ml of Ag. The Effective Lethal Concentration for the microalgae *Rhodomonas sp.* was 4 µg/ml of Ag. In fungi, the MIC was 42 µg/ml for *C. albicans*, and, 20 µg/ml for *F. oxysporum*. The Lethal Dose in all animal and cancer cells ranged from 10 to 12 µg/ml of Ag. In summary, AgNPs exerted inhibitory effects in all biological models tested in vitro (figure 1). We also performed an exhaustive literature revision regarding the toxicity of AgNPs in organisms of different taxonomic groups (included in figure 2). We only considered those studies with an adequate characterization of the AgNPs. AgNPs toxicity is not related to the complexity of the cell (structural or physiological) [11].

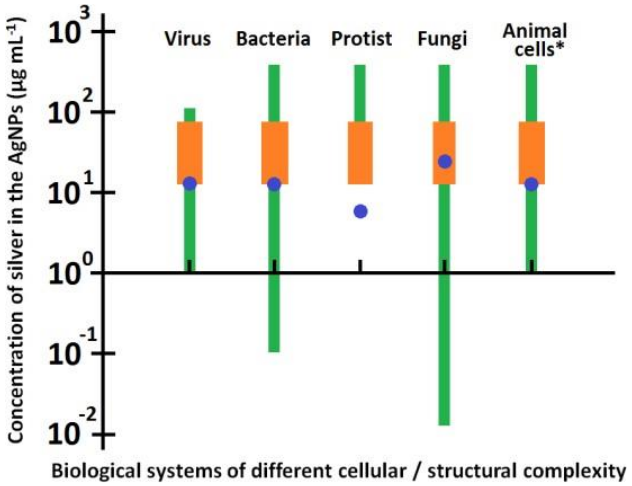

Figure 2. Inhibition concentration range (green slim bars) of the AgNPs, as reported in different works. Most studies show an inhibitory concentration of 10^1 µg/ml of silver – AgNPs- (orange boxes). The blue dots show the concentration values determined in our study, using the same nanomaterial (PVP-AgNPs) for all the biological systems tested. Modified from Vazquez-Muñoz et al [11]

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4 Conclusions

To the best of our knowledge, no other single nanomaterial has been tested in such a wide spectrum of BS of different levels of cellular/structural complexity, ranging from virus to human cell lines. Our experimental analysis showed that viruses and cells of different complexity are inhibited *in vitro* at similar concentrations of silver (10^1 µg/ml). Also, the meta-analysis supports our results. Despite the differences between the different AgNPs, the lethal concentration in the majority of the studies for both viruses and cells occurs within narrow concentration range around 10^1 µg/ml of Ag.

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