SILVER NANOPARTICLES: MORPHOLOGY, ADMINISTRATION AND HEALTH RISKS

Nikolay Zahariev*, Velichka Andonova, Dimitar Penkov, Margarita Kassarova
Medical University of Plovdiv, Faculty of Pharmacy, Plovdiv 4002, Bulgaria
E-mail: nikizahariev93@gmail.com

ABSTRACT

In recent decades, the applying of nano- and micro- carriers in the pharmaceutical technology is growing rapidly. They are used to protect the drug from the harmful effects of the environment, to increase the rate and extent of dissolution and thus they improve its bioavailability or provide controlled release of the drug substance for a period of time, at the desired location. A number of metallic, polymeric or inorganic derivatives are used to obtain the nanocarriers. Silver nanoparticles (Ag-NPs), with their antifungal, antibacterial and antiseptic properties, are of particular interest to the pharmaceutical industry. Several studies show that in addition to their beneficial effects on the human body, they can also cause side and toxic effects. The severity of these conditions is most often associated with the path of introduction of the Ag-NPs, their morphology, the dose and duration of the treatment. It is also of great significance the effect of such a production on the environment. The aim of this review is to evaluate the benefit – risk ratio of the use of Ag-NPs in the pharmaceutical practice.

Keywords: Silver, argyrosis (argyria), toxicity of silver nanoparticles

INTRODUCTION

Silver is a precious metal that occurs in nature as a native element or as a mineral, associated with other elements: as a primary constituent in silver minerals; as a natural alloy with other metals; and, as a trace in the ores of other metals (1). It is the 47^{th} element of the periodic table with relative atomic mass of 107.868. In the past, this metal has been used in daily life for manufacture of various household tools, jewelry, coins, clothing, building materials, as well as a disinfectant. Silver salts have been used in the medicine for treatment of psychiatric disorders, nicotine dependence, gastroenteritis and infectious diseases such as syphilis and gonorrhea (2). Currently, Ag-NPs are entering deeper into human life. To prevent food poisoning, silver ions are added to cutting boards, cutlery, refrigerators, surface disinfectants and others. In the textile industry, nanosilver is impregnated in various tissues in order to disinfect and prevent the development of bacterial skin rashes. The antimicrobial activity against a large number of pathogenic microorganisms is one of the reasons for the introduction of Ag-NPs in the pharmaceutical industry and medicine. For example, modern catheters containing silver ions are widely used because of the antimicrobial activity of the silver (3). Loutfy *et al*. have provided a flexible platform for enhancing anticancerous activity of 6-mercapopurine against human breast cancer using Ag-NPs and gold NPs (4). In another survey Yang *et al*. have studied the antitumor activity of 9–aminoacridine loaded Ag-NPs (5). Their results suggest that Ag-NPs may serve as slow-release drug carriers, which is important in antitumor drug delivery. However, we should ask ourselves some questions: how well we studied and predicted the potential risk of their application; are there enough reliable and specific tests to determine their toxicity; is there a balance between the benefits and the risks of their use; what is their long term impact on the human body. Ostad *et al*. have studied the cytotoxicity of Ag+ and Ag-NPs in parent and tamoxifen-resistant *T47D* human breast cancer cell lines in comparison (6). The results revealed that at non-cytotoxic concentrations of Ag+, Ag-NPs, and tamoxifen, the combinationof Ag+ -tamoxifen and Ag-NPs-tamoxifen is still cytotoxic to tamoxifen-resistance cells. Although these results may be of great potential benefit in chemotherapy of breast cancer, they also showed that by using NPs in non-toxic concentrations, this approach is much concerned about potential hazards of prevalent use of metallic NPs.

Generally, the behavior of the NPs is relatively different from that of the larger particles of the identical material. There is a correlation between the particle size and their toxicity: the smaller the size of the NPs, the more pronounced is their toxicity, due to increased contact surface (7). The uniqueness of the NPs is due to their ability to penetrate deeply into the respiratory system, skin and mucous membranes, and thus better distribute in the body fluids. Drug nanocarriers could cause side effects or toxicity because of the origin of the NPs and the place of their application. On the other hand, due to their high specific surface area, they may contain traces of other substances (be contaminated), which can cause a risk in their application. To evaluate the potential risk of using NPs in the field of nanomedicine, we will present some examples, related to Ag-NPs.

Metallic silver, silver ions, colloidal silver, nanosilver

Silver is found in various forms in nature and in living organisms. The most common of them are metallic silver, silver salts (i.e. ionized silver), silver complexes and colloidal silver (8). The metallic silver is dissolved in an acidic medium, thus forming silver salts. In the aqueous solutions of soluble silver salts, silver is in the form of silver cations (Ag^+) and they can form complexes with many organic ligands. Colloidal silver is a colloid compound of Ag-containing particles dispersed in water, and the particle size is from 1 nm to 1 micron. Colloidal silver can be found in 2 forms: superfine metal form or in the form of insoluble silver compounds, dispersed in a solution, which acquires different turbidity (8).

Nanosilver represents silver nano-sized particles, measured in nm. The term is most often associated with colloidal silver. There are many commercial products, one of them named "Nano-Silver", a commercial designation of Ag-NPs in the form of a water suspension (9). These silver particles have a size between 5 and 50 nm. The greater part is in the form of metallic silver, while the rest is in an ionized form. It has a large contact surface and better porosity, compared to the commercial silver; this is why it has a more pronounced effect at lower concentrations (8,9). Due to the small particle sizes, there is a maximal contact surface, which is the reason for the high efficiency. As a result, a minimum concentration of the Nano-Silver provides greater efficacy, compared to colloidal silver with much higher concentration. Nano-Silver is characterized with a high percentage of silver in metallic form. This is important because in the stomach, ionized silver is converted to silver chloride under the influence of the hydrochloric acid. The silver chloride has a low solubility and it is less active, compared to metal silver (8,9). Another commercially available product is SilverSol™, desined by Nano Silver Manufacturing Sdn Bhd. (Malaysia) (10). SilverSol™ is the colorless, tasteless, and odorless solution suspended in the distilled water as the state of colloid. It is produced from pure silver (Ag 99.99%) through high electrical voltage. The particle size is between 3-5 nm as compared to common colloidal silver which is 8 nm - 10 μm.

Silver atoms can form under certain conditions the so-called silver clusters. Later they aggregate and convert into NPs (8).

Mechanism of action of silver NPs

In an extensive review Lara *et al.* have discussed recent advances in the understanding of the biocidal mechanisms of action of Ag-NPs (11). Due to its physicochemical properties, Ag-NPs can easily pass through cell membranes in the body, connect with the biologically active molecules and achieve its effect. The exact mechanism of the antibacterial action of the NPs has not been yet elucidated. There are several theories explaining the bactericidal effect. Ag-NPs possess the ability to bind specific proteins on the bacteria cell wall. The formation of Ag-NPs-protein complex activates

intracellular signaling pathways that cause disorders in the permeability of the bacterial cell membrane and cell death (12,13). Another possible mechanism is the formation of free radicals from the Ag-NPs (13). Most sensitive to nanosilver are the mitochondria (13,14). A study found that Ag-NPs accumulate outside the mitochondria and cause mitochondrial damage (14). As a result, reactive oxygen species (ROS) are released. According P.V. AshaRani *et al.* (15) the destruction of the mitochondrial respiratory chain increases the production of ROS, impairs the production of ATP, causing DNA damage. Silver NPs can release silver ions, reacting with the S^{2-} or SH^{-} groups of some metabolic enzymes (16,17). Impaired metabolism leads to cell death. The interaction of the Ag-NPs with the DNA molecules causes cell division disturbances, affecting the replication process (18). It has been found that Ag-NPs can modulate signal transduction in the bacterial cell through modulating the phosphotyrosine profile of putative bacterial peptides and thus to inhibit the bacterial cell growth (19).

Toxicity of silver NPs

A number of studies have shown that in addition to their antibacterial activity, Ag-NPs exhibit an extreme toxicity on nature and human body. Ecologists report that industrial waste emit in nature tons of silver, as soluble silver compounds. In these compounds, Ag is in the form of silver ions, which are responsible for the toxic effects like: permanent discoloration of the skin (argyria) and eyes (argyrosis), damage to the liver and kidneys, gastrointestinal tract, respiratory and blood system (8).

Ag-NPs can exert their toxic effect on the human body through several mechanisms. They may exhibit their toxicity by changing the cell wall permeability; disrupt the K / Na concentration by formation of a silver ion; and cause disorders in the mitochondrial activity (20). Ag-NPs induce changes on proliferation and cytokine expression of the mononuclear cells (21), also affecting the male reproductive system. Liver is one of the most sensitive organs regarding the effects of the Ag-NPs. Several *in vivo* and *in vitro* studies support the assumption for the toxic potential of the Ag-NPs in regard to the liver. In an *in vivo* study, Almofti MR *et al.* (22) observed some metabolic disorders and increased mitochondrial permeability. The survey was conducted on mice, in a dose range from 222 to 362 mg/kg/day Ag-NPs. On the other hand, an *in vitro* study demonstrates that even a very low concentration of Ag-NPs can cause mitochondrial damage (23).

Routes of penetration and toxic effects of Ag-NPs

1. Gastrointestinal tract (GI tract)

GI tract is the main entry route for macromolecules in the body. The absorption of the particles depends on the particle size, charge, and dose. The smaller the particles are, the faster they are absorbed. Silver NPs, due to their extremely small size are easily absorbed in the GI tract. A number of studies investigate the toxic effects of the Ag-NPs after oral administration.

Kim SC *et al*. (24) investigated the impact of Ag-NPs with size of 60 nm and 0.5% carboxymethyl cellulose in a dose of 125 mg/kg. The study was conducted on rats for a period of 90 days. An increase of cholesterol and cholestatic enzymes (alkaline phosphatase) was established, which leads to the development of biliary hyperplasia. No toxic effects on the kidneys were found, although there was an accumulation.

In another *in vivo* study, conducted by Park EJ *et al*. (25), rats were treated with Ag-NPs in a dosage regimen 0.25-1.0 mg/kg for 28 days. Minimal dose-dependent increase in the serum pro-inflammatory (IL-1, IL-6, IL-12), and anti-inflammatory (IL-10, TGF-beta) cytokines was found.

Bouwmeester H *et al.* (26) investigated the impact of Ag-NPs on human epithelial colorectal adenocarcinoma cells. Treating the cell culture *Caco-2* with Ag-NPs in

concentration 50 mcg, for 24 hours leads to dissociation of 6 to 17% of the NPs, which pass into ionized form. Using the same cell line *Caco-2* and *HepG2*, Lamb JG *et al*. (27) found that after 24 hours of treatment Ag-NPs, in concentration 1-10 μg/ml, cause cytotoxicity. The established LD50 values have been ~ 4 μg silver/ml for *HepG2* and 5 μg/ml for *Caco-2* cells.

The absorption of silver ions through the cell membranes and the gastrointestinal mucosa is due to the presence of free, ionized silver ions. Due to their high biological activity, silver ions bind organic precipitates contained in food (phytate, fibers etc.) and inorganic ions, such as chlorides and phosphates, causing an increased absorption. Silver ions are absorbed into the systemic circulation from food and water, by inhalation and through percutaneous absorption. Ag^+ binds strongly to albumins, macro globulins, metallothionein and is metabolized to all tissues, except brain and central nervous system. High risk of intoxication with silver was observed in the uncontrolled use of colloidal silver products containing high concentrations of ionized silver. Some of the major effects, associated with heavy deposition of insoluble silver precipitates in the dermis and cornea are argyria and argyrosis. High oral intake of silver also causes corrosive condition in the oral cavity and GI tract, pain, even death (28, 29).

2. Inhalation

Silver can penetrate the body through the respiratory tract. Preparations, containing Ag-NPs, colloidal silver, silver oxide or silver nitrate use this road of penetration in the body (30). Comprehensive studies show that silver can enter through the nasal mucosa or the respiratory membranes. Passing through, it accumulates in the mucus secretions or the pulmonary surfactant and thus silver ions can be absorbed through the alveolar epithelium. Inhalation of ionized silver particles can cause bronchitis, squamous metaplasia, and pigmentation of the respiratory tract, looking like anthracosis or siderosis (31). This is due to the fact that the ionized silver has a more pronounced toxic effect compared to the metal form. In a study conducted by Stebounova *et al*. (32), it has been found trough histological and spectral analyses, that commercially available nanosilver shows a minimal risk of development of pulmonary inflammation and pulmonary cytotoxicity. The study was conducted on rats, inhaled with nanosilver (3.3 mg/m^3, 4 hours/day for 10 days), with particle size from 2 to 5 nm. Thinning of the alveolar membrane and granulomatous changes occur in prolonged contact with Ag-NPs, but deaths have not been established. The authors have concluded that longer term exposures with higher lung burdens of nanosilver are needed to ensure that there are no chronic effects and to evaluate possible translocation to other organs.

3. Dermal road of penetration and percutaneous absorption

The majority of the products containing silver and silver compounds with antibiotic purpose are intended for topical administration. Clinical and experimental studies have shown that percutaneous silver absorption is exceptionally low. Epidermal keratin and phospholipids constituting the epidermal barrier did not allow the absorption of silver and other metals through the skin. This is due to the presence of sulfhydryl groups, binding irreversibly silver ions, as well as other metal ions, thus blocking their penetration (29). Frequent use of metallic silver, silver threads or silver impregnated fabrics, with hygienic purpose can lead to an increased percutaneous absorption, elevated serum levels of silver and even accumulation of silver precipitates in the skin (at chronic exposure). However, the use of silver preparations in textiles, *etc*., even when the skin is warm and moist, could not lead to the development of argyria. According to an *in vivo* study on rats (100 μg/ml, 3-7 days), Ag-NPs do not lead to any external changes and observable inflammatory responses in animal skin, but it can alter the level of some biomarkers related to liver function in blood serum of samples (33).

Conclusion

This paper aims to show the potential risk of usage of Ag-NPs in pharmaceutical practice. The benefits from nano particulate drug delivery systems are undoubted. They may protect the drug from undesirable effects of the environment, providing a targeted drug delivery with a controlled release and reduced side effects. There are many studies which show the antibacterial, the antifungal, the antiproliferative and antitumor activity of silver compounds, in particular to the Ag-NPs. And although the results cited in this study are only a small part of all studies conducted in recent years, they undoubtedly prove the potential health risk from the application of Ag-NPs. The relevant factors which most greatly affect the toxicity of these NPs are size, shape, surface morphology, the presence of functional groups, solubility or more accurately what part of the silver is ionized, the dose administered for the period of treatment and the route of administration. The combination of the advantages provided from nanoparticulate drug delivery systems and these from Ag-NPs should be used with high attention in order to minimize the potential side effects of these systems. Specific investigations are required in terms of the dosage used for certain period and route of administration. The attention has to be pointed out not only trough acute toxicity but also a prolonged retention in the biological fluids and potential long term toxicity.

References

1. Pryor M. R. Mineral Processing. 3rd Ed. Springer Science & Business Media 2012; pp. 736.
2. Drake P.L., Hazelwood K.J. Exposure-Related Health Effects of Silver and Silver Compounds: A Review. Ann. Occup. Hyg. 2005; 49(7): 575-85.
3. Samuel U., Guggenbichler J. P. Prevention of catheter-related infection: the potential of a new nano-silver impregnated catheter. Int. J. Antimicrob. Agents 2004; 23(1):75-8.
4. Loutfy S. A., Mohamed M. B., Abdel-GhaniN. T., Al-AnsaryN., Abdulla W. A., El-Borady O. M., Hussein Y., Eldin M. H. Metallic Nanomaterials as Drug Carriers to Decrease Side Effects of Chemotherapy (InVitro: Cytotoxicity Study). Journal of Nanopharmaceutics and Drug Delivery 2013; 2(1): doi:10.1166/jnd.2013.1010.
5. Yang J, Wang H, Wang Z, Tan X, Song C, Zhang R, Li J, Cui Y. Interaction between antitumor drug and silver nanoparticles: combined fluorescence and surface enhanced Raman scattering study. Chin. Opt. Lett. 2009; 07(10): 894-7.
6. Ostad SN, Dehnad S, Nazari ZE, Fini ST, Mokhtari N, Shakibaie M, Shahverdi AR. Cytotoxic Activities of Silver Nanoparticles and Silver Ions in Parent and Tamoxifen-Resistant T47D Human Breast Cancer Cells and Their Combination Effects with Tamoxifen against Resistant Cells. Avicenna J Med Biotech 2010; 2(4): 187-96.
7. Vishwakarma V, Samal SS, Manoharan N. Safety and risk Associated with Nanoparticles-Review. Journal of minerals & Materials Characterization & Engineering 2010; 9(5): 455-9.
8. Panyola NR, Peňa-Méndez EM, Havel J. Silver or silver nanoparticles: a hazardous threat to the environment and human health? J Appl Biomed 2008; 6:117-29.
9. Nano-silver. Available from: http://www.nano-silver.com/ [cited: 14th June 2016].
10. SilverSol™. Available from: http://www.nanosilver.com.my/portfolio-item/silversol/ [cited: 14th June 2016].
11. Lara HH, Garza-Treviño EN, Ixtepan-Turrent L, Singh DK. Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. Journal of Nanobiotechnology. 2011; 9:30. doi:10.1186/1477-3155-9-30.

12. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J. Colloid Interface Sci. 2004; 275: 177–82.
13. McShan D, Ray PC, Yu H. Molecular toxicity mechanism of nanosilver - Review Article. Journal of Food and Drug Analysis 2014; 22(1): 116-27.
14. Bressan E., Ferroni L., Gardin C., et al. Silver nanoparticles and mitochondrial interactions. Int J Dentistry 2013; Article ID 312747.
15. AshaRani P.V., Mun G.K., Hande M.P., et al. Cytotoxicity and genotoxicity of nanosilver nanoparticles in human cells. ASC Nano 2009: 3: 279-90.
16. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J. Biomed. Mater. Res. 2008; 52: 662–8.
17. Matsumura Y, Yoshikata K, Kunisaki S, Tsuchido T. Mode of bacterial action of silver zeolite and its comparison with that of silver nitrate. Appl. Environ. Microbiol. 2003; 69: 4278–81.
18. Lim H., Asharani P., Handle P. Enhanced genotoxicity of silver nanoparticles in DnA repair deficient mammalian cells. Front Genet 2012; 3: 104.
19. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. Characterisation of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology 2007; 18: 1–9.
20. Kone BC, Kaleta M, Gullaus SR. Silver ion cell membrane K^+ and Na^+ permeability in the renal proximal tubule: reversal by the thiol reagents. J. Membr. Biol. 1988; 102: 11-19.
21. Shin SH, MK Ye, Kim HS, Kang HS. The effects of nano-silver on the proliferation and cytokine expression by peripheral blood mononuclear cells. Int. Immunopharmacology 2007; 7(13): 1813-8.
22. Almofti MR, Ichirawa T, Yamashita K, Terada H, Shiconahara X. Silver ion induces cyclosporine A-insensitive permeability, trancition in rat liver, mitochondria and release of apoptogenic cytochrome C. J. Biochem.2003; 134: 43-9
23. Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlage JJ. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol In Vitro 2005; 19(7): 975-83.
24. Kim SC, Chen DR, et al. A nanoparticle dispersion method for in vitro and in vivo nanotoxicity study. Nanotoxicology. 2010; 4(1): 42–51.
25. Park EJ, Bae E, et al. Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles. Environmental Toxicology and Pharmacology. 2010; 30(2): 162–8.
26. Bouwmeester H, Poortman J, et al. Characterization of translocation of silver nanoparticles and effects on whole-genome gene expression using an in vitro intestinal epithelium coculture model. ACS Nano. 2011; 5(5): 4091–103.
27. Lamb JG, Hathaway LB, et al. Nanosilver particle effects on drug metabolism in vitro. Drug Metab. Dispos. 2010; 38(12): 2246–51.
28. Fung M.C., Bowen D. L. Silver products for medical indications: risk-benefit assessment. Journal of Toxicology-Clinical Toxicology 1996; 34(1): 119-26.
29. Lansdown ABG. A Pharmacological and Toxicological Profile of Silver as an Antimicrobial Agent in Medical devices. Advances in Pharmacological Sciences 2010; Article ID 910686.
30. Fung M.C, Weintraub M., Bowen D.L. Colloidial silver proteins marketed as health supplements. Journal of the American Medical Association 1995; 274(15): 1196-7.
31. Seiffert J, Hussain F, Wiegman C, Li F, Bey L, Baker W, Porter A, Ryan MP, Chang Y, Gow A, Zhang J, Zhu J, Tetley TD, Chung KF. Pulmonary Toxicity of Instilled Silver Nanoparticles:

Influence of Size, Coating and Rat Strain. PLoS One. 2015; 10(3):e0119726. doi: 10.1371/journal.pone.0119726. eCollection 2015.
32. Stebounova LV, Adamacakova-Dodd A, Kim JS, park H, O'Shanghnessy P, Grassian VA, Thorne PS. Nanosilver induces minimal lung toxicity or inflammation in a subacute murine inhalation model. Particle and Fiber Toxicology 2011; 8:5.
33. Mehran Arabi, Parisa Yarmohamadi Samani. A study of nanosilver dermal toxicity in mice balb/c. Journal of Pharmaceutical and Health Sciences 2013; 2(1): 33-7.