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Review

Silver Nanoparticles as Potential Antiviral Agents

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Table 1. Antiviral metal nanoparticles.

Virus	Family	Metal Nanoparticle Composition (size)	Mechanism of Action	References
Human immunodeficiency virus type 1 (HIV-1)	Retroviridae	PVP-coated silver nanoparticles (1–10 nm)	Interaction with gp120	[38–40]
Herpes simplex virus type 1 (HSV-1)	Herpesviridae	MES-coated silver and gold nanoparticles (4 nm)	Competition for the binding of the virus to the cell	[43,44]
Respiratory syncytial virus	Paramyxoviridae	PVP-coated silver nanoparticles (69 nm +/− 3 nm)	Interference with viral attachment	[42]
Monkeypox virus	Poxviridae	Silver nanoparticles and polysaccharide-coated Silver nanoparticles (10–80 nm)	Block of virus-host cell binding and penetration	[45]
Influenza virus	Orthomyxoviridae	Sialic-acid functionalized gold nanoparticles (14 nm)	Inhibition of virus binding to the plasma membrane	[46]
Tacaribe virus (TCRV)	Arenaviridae	Silver nanoparticles and polysaccharide-coated Silver nanoparticles (10 nm)	Inactivation of virus particles prior to entry	[47]
Hepatitis B virus (HBV)	Hepadnaviridae	Silver nanoparticles; (10–50 nm)	Interaction with double-stranded DNA and/or binding with viral particles	[41]

5. Conclusions

In the crusade toward the development of drugs for the therapy of viral diseases, the emergence of resistant viral strains and adverse side effects associated with a prolonged use represent huge obstacles that are difficult to circumvent. Therefore, multidisciplinary research efforts, integrated with classical epidemiology and clinical approaches, are crucial for the development of improved antivirals through alternative strategies. Nanotechnology has emerged giving the opportunity to re-explore biological properties of known antimicrobial compounds, such as metals, by the manipulation of their sizes. Metal nanoparticles, especially the ones produced with silver or gold, have proven to exhibit virucidal activity against a broad-spectrum of viruses, and surely to reduce viral infectivity of cultured cells. In most cases, a direct interaction between the nanoparticle and the virus surface proteins could be demonstrated or hypothesized. The intriguing problem to be solved is to understand the exact site of interaction and how to modify the nanoparticle surface characteristics for a broader and more effective use. Besides the direct interaction with viral surface glycoproteins, metal nanoparticles may gain access into the cell and exert their antiviral activity through interactions with the viral genome (DNA or RNA). Furthermore, the intracellular compartment of an infected cell is overcrowded by virally

encoded and host cellular factors that are needed to allow viral replication and a proper production of progeny virions. The interaction of metal nanoparticles with these factors, which are the key to an efficient viral replication, may also represent a further mechanism of action (Figure 2).

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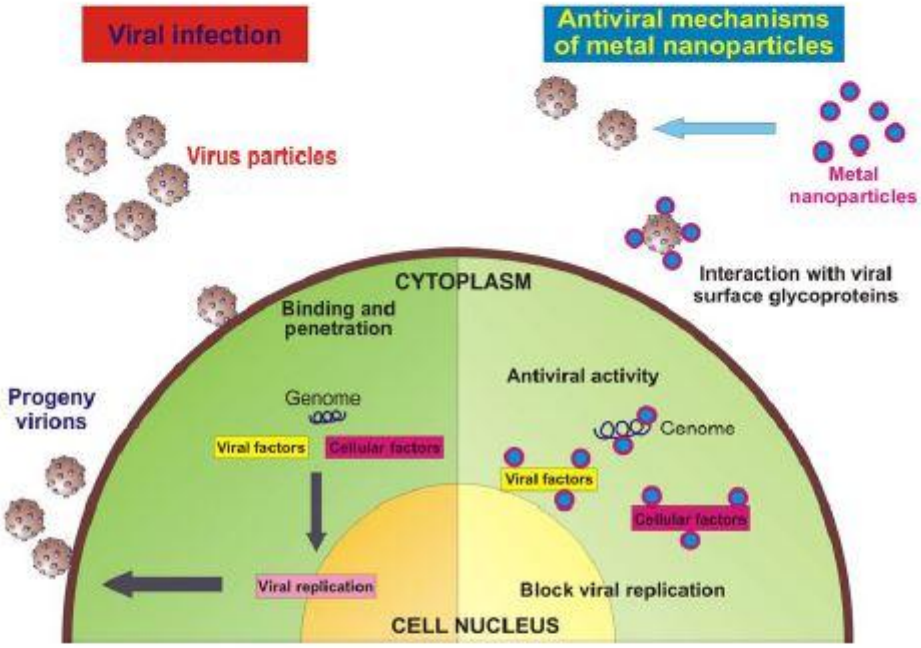
Figure 2. Schematic model of a virus infecting an eukaryotic cell and antiviral mechanism of metal nanoparticles.



Most of the published literature describes the antiviral activity of silver or gold nanoparticles against enveloped viruses, with both a DNA or an RNA genome. Considering that one of the main arguments toward the efficacy of the analysed nanoparticles is the fact that they in virtue of their shape and size, can interact with virus particles with a well-defined spatial arrangement, the possibility of metal nanoparticles being active against naked viruses seems appealing. Moreover, it has been already proven that both silver and gold nanoparticles may be used as a core material. However, no reports are yet available for the use of other metals, but the future holds many surprises, especially considering that the capping molecules that could be investigated are virtually unlimited.

Nonetheless, for metal nanoparticles to be used in therapeutic or prophylactic treatment regimens, it is critical to understand the *in vivo* toxicity and potential for long-term sequelae associated with the exposure to these compounds. Additional research is needed to determine how to safely design, use, and dispose products containing metal nanomaterials without creating new risk to humans or the environment.

Conflict of Interest

The authors declare no conflict of interest.

38. Sun, R.W.; Chen, R.; Chung, N.P.; Ho, C.M.; Lin, C.L.; Che, C.M. Silver nanoparticles fabricated in Hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells. *Chem. Commun. (Camb)* **2005**, *40*, 5059–5061.
39. Lara, H.H.; Ayala-Nuñez, N.V.; Ixtepan-Turrent, L.; Rodriguez-Padilla, C. Mode of antiviral action of silver nanoparticles against HIV-1. *J. Nanobiotechnol.* **2010**, *8*, 1–10.
40. Lara, H.H.; Ixtepan-Turrent, L.; Garza-Treviño, E.N.; Rodriguez-Padilla, C. PVP-coated silver nanoparticles block the transmission of cell-free and cell-associated HIV-1 in human cervical culture. *J. Nanobiotechnol.* **2010**, *8*, 15–25.
41. Lu, L.; Sun, R.W.; Chen, R.; Hui, C.K.; Ho, C.M.; Luk, J.M.; Lau, G.K.; Che, C.M. Silver nanoparticles inhibit hepatitis B virus replication. *Antivir. Ther.* **2008**, *13*, 253–262.
42. Sun, L.; Singh, A.K.; Vig, K.; Pillai, S.; Shreekumar, R.; Singh, S.R. Silver nanoparticles inhibit replication of respiratory sincitial virus. *J. Biomed. Biotechnol.* **2008**, *4*, 149–158.
43. Baram-Pinto, D.; Shukla, S.; Perkas, N.; Gedanken, A.; Sarid, R. Inhibition of herpes simplex virus type 1 infection by silver nanoparticles capped with mercaptoethane sulfonate. *Bioconjug. Chem.* **2009**, *20*, 1497–1502.
44. Baram-Pinto, D.; Shukla, S.; Gedanken, A.; Sarid, R. Inhibition of HSV-1 attachment, entry, and cell-to-cell spread by functionalized multivalent gold nanoparticles. *Small* **2010**, *6*, 1044–1050.
45. Rogers, J.V.; Parkinson, C.V.; Choi, Y.W.; Speshock, J.L.; Hussain, S.M. A preliminary assessment of silver nanoparticles inhibition of monkeypox virus plaque formation. *Nanoscale Res. Lett.* **2008**, *3*, 129–133.
46. Papp, I.; Sieben, C.; Ludwig, K.; Roskamp, M.; Böttcher, C.; Schlecht, S.; Herrmann, A.; Haag, R. Inhibition of influenza virus infection by multivalent sialic-acid-functionalized gold nanoparticles. *Small* **2010**, *6*, 2900–2906.
47. Speshock, J.L.; Murdock, R.C.; Braydich-Stolle, L.K.; Schrand, A.M.; Hussain, S.M. Interaction of silver nanoparticles with Tacaribe virus. *J. Nanobiotechnol.* **2010**, *8*, 19–27.

Silver nanoparticles interactions with the immune system: implications for health and disease

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3. Silver nanoparticles

In nanotechnology, a nanoparticle is defined as a material with dimensions and tolerance limits of 0.1-100nm that behaves as a whole unit in terms of its transport, properties and unique characteristics.
Metallic nanoparticles have unique optical, electrical and biological properties that have attracted significant attention due to their potential use in many applications, such as catalysis, biosensing, drug delivery and nanodevice fabrication. Capped silver nanoparticles (AgNPs) have many biomedical applications due to its excellent biocompatibility and antibacterial properties. It has been reported that silver nanoparticles interact with virus, bacteria, and the immune system, being the reason why in this chapter we will explain how the size, shape and composition of silver nanoparticles can have a significant effect on their efficacy and have to be kept in mind when using bioconjugates.

Silver Nanoparticles: No Threat to the Environment

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Abstract:

Silver is an effective germ fighter and silver nanoparticles are widely recognized as being especially effective because of their enormously high surface area. Due to the large number of manufacturers using silver nanoparticles in their products, some concern has arisen about the effects on the environment when these products are disposed of or washed. This report will demonstrate that silver nanoparticles do not remain "nanosize" when they come in contact with normal environmental samples, such as soil and water, but they agglomerate to form much larger, much less biologically effective, silver particles which are non-toxic, non-ionic and have no history of being harmful to the environment or aquatic life. Furthermore, there is no possibility that silver nanoparticles can ever form silver ions, except in the presence of strong oxidizing substances.

FULL PAPER *Toxicology*

An Evaluation of Acute Toxicity of Colloidal Silver Nanoparticles

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ABSTRACT. Tests for acute oral toxicity, eye irritation, corrosion and dermal toxicity of colloidal silver nanoparticles (AgNPs) were conducted in laboratory animals following OECD guidelines. Oral administration of AgNPs at a limited dose of 5,000 mg/kg produced neither mortality nor acute toxic signs throughout the observation period. Percentage of body weight gain of the mice showed no significant difference between control and treatment groups. In the hematological analysis, there was no significant difference between mice treated with AgNPs and controls. Blood chemistry analysis also showed no differences in any of the parameter examined. There was neither any gross lesion nor histopathological change observed in various organs. The results indicated that the LD_{50} of colloidal AgNPs is greater than 5,000 mg/kg body weight. In acute eye irritation and corrosion study, no mortality and toxic signs were observed when various doses of colloidal AgNPs were instilled in guinea pig eyes during 72 hr observation period. However, the instillation of AgNPs at 5,000 ppm produced transient eye irritation during early 24 hr observation time. No any gross abnormality was noted in the skins of the guinea pigs exposed to various doses of colloidal AgNPs. In addition, no significant AgNPs exposure relating to dermal tissue changes was observed microscopically. In summary, these findings of all toxicity tests in this study suggest that colloidal AgNPs could be relatively safe when administered to oral, eye and skin of the animal models for short periods of time.

KEY WORDS: acute toxicity, colloidal silver nanoparticles, dermal, eye, oral.

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RESULTS

Clinical and general signs: In all acute toxicity tests, no death was recorded in the 14 days of observation period in all control and treated animals. The animals did not show any significant changes in the general appearance during the observation period. There were no significant differences in the percentage of weight gain between the control and treatment groups of both male and female mice given 5,000 mg/kg of the colloidal AgNPs orally (Table 1). Percentage of body weight gain AgNPs in male mice on the first day decreased. Moreover, some mice from this group showed bite wounds in scrotal areas and tails. No significant changes in water / food consumption, % weight gain and behavior of guinea pigs in acute eye and dermal toxicity test were also observed during the observation time (data not shown).

Hematology and clinical chemistry: The hematological analysis in acute oral toxicity test showed no significant changes of RBC, Hb, Ht, MCV, MCH, MCHC, platelets and WBC in the male and female treatment groups compared to the control groups. The leukocyte differential count showed no significant difference between the control and treated groups (Table 3). There were no significant differences in any of the biochemical parameters examined in either the control or treated group of the male and female mice (Table 4).

Gross and histopathology: There was no significant lesion on gross findings in any observation time of all acute toxicity tests. In acute eye irritation and corrosion test, some animals from 5,000 ppm AgNPs treated group showed grade 1 of conjunctivae irritation, which some blood vessels hyperemia in conjunctivae were observed during the first 24

hr observation time (Fig. 3). However, no ocular reaction was found in all treated animals after 48 hr post-exposure (Table 2). Histopathological examination of various organs in the control and treated animals showed no remarkable lesions that could be attributed to the effect of oral and dermal exposure of AgNPs at all observation times. Accumulation of free aggregated AgNPs was found on the epidermal layers of some 100,000 ppm treated animals (Fig. 4) and the mucosal areas of gastrointestinal tracts of the treated mice after 24 hr post-exposure. However, no evidence of penetration or infiltration of AgNPs was observed in all accumulated areas.

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ORIGINAL RESEARCH

Antitumor activity of silver nanoparticles in Dalton's lymphoma ascites tumor model

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Abstract: Nanomedicine concerns the use of precision-engineered nanomaterials to develop novel therapeutic and diagnostic modalities for human use. The present study demonstrates the efficacy of biologically synthesized silver nanoparticles (AgNPs) as an antitumor agent using Dalton's lymphoma ascites (DLA) cell lines in vitro and in vivo. The AgNPs showed dose-dependent cytotoxicity against DLA cells through activation of the caspase 3 enzyme, leading to induction of apoptosis which was further confirmed through resulting nuclear fragmentation. Acute toxicity, ie, convulsions, hyperactivity and chronic toxicity such as increased body weight and abnormal hematologic parameters did not occur. AgNPs significantly increased the survival time in the tumor mouse model by about 50% in comparison with tumor controls. AgNPs also decreased the volume of ascitic fluid in tumor-bearing mice by 65%, thereby returning body weight to normal. Elevated white blood cell and platelet counts in ascitic fluid from the tumor-bearing mice were brought to near-normal range. Histopathologic analysis of ascitic fluid showed a reduction in DLA cell count in tumor-bearing mice treated with AgNPs. These findings confirm the antitumor properties of AgNPs, and suggest that they may be a cost-effective alternative in the treatment of cancer and angiogenesis-related disorders.

Keywords: antitumor, silver nanoparticles, Dalton's lymphoma, ascites

vessels develop.[38] Tumor cells implanted into the peritoneal cavity secrete vascular permeability factor and thereby render the microvasculature supplying the peritoneal lining tissues hyperpermeable.[39] With respect to vascular hyperpermeability, the ascites tumor model used in the present study resembles a solid tumor model, with angiogenesis and generation of a connective tissue stroma. AgNPs that have been proven to delay tumor progression in DLA cell lines and tumor models in vivo may have a potent antipermeability effect by inhibiting tubular formation in growth factor- and advanced glycation end product-induced vascular permeability and cytotoxic effects that inhibit existence of tumor cells, which may be due to their potent activation of the caspase enzyme, as demonstrated in this study. The role of AgNP in inhibiting DLA cell viability and proliferation will be similar to their potential to inhibit the permeability of endothelial cells by inactivating Src kinases which have been proven to have a role in retinal therapies.[16] The pathways by which AgNPs inhibit the pathway mediating cell proliferation and viability have yet to be explored.

AgNPs serve as antitumor agents by decreasing progressive development of tumor cells. This may be due to their inhibitory activities in several signaling cascades responsible for the development and pathogenesis of the disease which are as yet not understood.

Taken together, our data suggest that AgNPs can induce cytotoxic effects on DLA cells, inhibiting tumor progression and thereby effectively controlling disease progression without toxicity to normal cells.

Conclusion

It is predicted that nanotechnology will have a $3.1 trillion impact on the global economy by 2015.[40] The projected nanotechnology market is expected to be about US$25 billion (or €15 billion) in 2012.[41] Use of AgNPs should emerge as one of the novel approaches in cancer therapy and, when the molecular mechanism of targeting is better understood, the applications of AgNPs are likely to expand further.[42] The present study explores the potential antitumor activity of biologically synthesized AgNP in a DLA tumor system in vitro by activation of the caspase 3 enzyme which is known to have a potent inhibitory effect on disease progression in a mouse model, leading to a potent restorative effect in the treated tumor mice near to normal by reducing tumor volume and weight gain. These drug delivery systems are mainly developed according to their ability to differentiate between malignant and nonmalignant cells, making them a promising alternative to existing drugs. This type of targeting efficiency of AgNPs can be accomplished in future therapies using RGD peptide conjugation, which directly targets tumor cells without affecting normal cells. Thus, a study of the exact mechanism by which AgNPs inhibit signaling cascades responsible for the development and progression of the disease would be a tremendous breakthrough in the field of nanomedicine and make these agents an effective alternative in tumor and angiogenesis-related diseases.

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Disclosure

The authors report no conflict of interest in this research.

References

1. Yezhelyev MV, Gao X, Xing Y, Hajj AA, Nie S, Regan RMO. Emerging use of nanoparticles in diagnosis and treatment of breast cancer. *Lancet Oncol*. 2006;7:657–667.
2. Wagner V, Dullaart A, Bock AK, Zweck A. The emerging nanomedicine landscape. *Nat Biotechnol*. 2006;24:1211–1217.
3. Sahoo SK, Dilnawaz F, Krishnakumar S. Nanotechnology in ocular drug delivery. *Drug Discov Today*. 2008;13:144–151.
4. Silver S, Phung LT, Silver G. Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. *J Ind Microbiol Biotechnol*. 2006;33:627–634.
5. Klasen HJ. Historical review of the use of silver in the treatment of burns. I. Early uses. *Burns*. 2000;26:117–130.
6. Bhattacharya R, Mukherjee P. Biological properties of "naked" metal nanoparticles. *Adv Drug Deliv Rev*. 2008;60:1289–1306.
7. Kalishwaralal K, BarathManiKanth S, Pandian SRK, Deepak V, Gurunathan S. Silver nano – a trove for retinal therapies. *J Control Release*. Mar 29, 2010. [Epub ahead of print].
8. Lara HH, Ayala-Nuñez NV, Ixtepan-Turrent L, Rodriguez-Padilla C. Mode of antiviral action of silver nanoparticles against HIV-1. *J Nanobiotechnology*. 2010;8:1.
9. Sun RW, Rong C, Chung NPY, Ho CM, Lin CLS, Che CM. Silver nanoparticles fabricated in Hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells. *Chem Commun (Camb)*. 2005;28:5059–5061.
10. Lu L, Sun RW, Chen R, et al. Silver nanoparticles inhibit hepatitis B virus replication. *Antivir Ther*. 2008;13:253–262.
11. Sun L, Singh AK, Vig K, Pillai SR, Singh SR. Silver nanoparticles inhibit replication of respiratory syncytial virus. *J Biomed Biotechnol*. 2008;4:149–158.
12. Baram-Pinto D, Shukla S, Perkas N, Gedanken A, Sarid R. Inhibition of herpes simplex virus type 1 infection by silver nanoparticles capped with mercapto ethane sulfonate. *Bioconjug Chem*. 2009;20:1497–1502.
13. Rogers JV, Parkinson CV, Choi YW, Speshock JL, Hussain SM. A preliminary assessment of silver nanoparticle inhibition of monkeypox virus plaque formation. *Nanoscale Research Letters*. 2008;3:129–133.
14. Kim JS, Kuk E, Yu KN, et al. Antimicrobial effects of silver nanoparticles. *Nanomedicine*. 2007;3:95–101.
15. Baker C, Pradhan A, Pakstis L, et al. Synthesis and antibacterial properties of silver nanoparticles. *J Nanosci Nanotechnol*. 2005;5:244–249.