Review Article

Ancient and Novel Forms of Silver in Medicine and Biomedicine

Alireza Ebrahiminezhad[1,2,3*], Mohammad Javad Raee[3], Zahra Manafi[4], Abdolreza Sotoodeh Jahromi[4], Younes Ghasemi[3*]

[1]Noncommunicable Diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran
[2]Department of Medical Biotechnology, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran
[3]Department of Pharmaceutical Biotechnology, School of Pharmacy and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[4]Department of Microbiology, Islamic Azad University, Jahrom Branch, Jahrom, Iran

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*Corresponding authors:
A. Ebrahiminezhad,
Y. Ghasemi,
Department of Pharmaceutical Biotechnology, School of Pharmacy and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
a_ebrahimi@sums.ac.ir
Ghasemiy@sums.ac.ir

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Abstract

Silver has been known and used as a potent antimicrobial and wound healing agent since ancient time. Silver compounds have had other ancient applications through which Greeks, Romans and Egyptians had used silver compounds as food and water preservative. Silver and silver-based antimicrobials were put away after the discovery of antibiotics. Meanwhile, with almost a century application of antibiotics, resistant microbial strains appeared and antibiotics are going to become less and less effective. Fortunately, our traditional weapon against microorganisms reemerged in a novel form to reclaim again. Silver nanoparticles (AgNPs) are well-known as potent and novel antimicrobial agents. AgNPs would disturb microbial growth through inhibiting the absorption of phosphate, collapsing the proton motive force, forming complexes with DNA, enzyme inactivation, as well as inhibition of glucose oxidation. Its follows attacking the respiratory chain, changing the permeability and potential of the cell membrane, and inducing bacteria into a viable but non-culturable (VBNC) state and eventually killing them.

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Introduction

Silver cations have been known as potent antimicrobial agents since ancient time and were used in burns, wounds and ulcers treatment(1). Silver was known to the Chaldeans as early as 4,000 B.C.E, and it was the third metal known to be used by the ancients, after gold and copper(2). Silver colloids have been used for numerous medical conditions, mostly empirically before realizing that microbes were the agents causing infection. The ancient Phoenicians, Greeks, Romans and Egyptians had used silver compounds as food and water preservative. The use of silver-based compounds for wound-healing and wound infection prevention and treatment were used by Hippocrates and Macedonians. They believed that treatment of ulcers with silver compounds not only could prevent infections but also promote the healing process. Even silver nitrate, an strong oxidative chemical, has had medical applications and was specified in a Roman pharmacopeia about 69 BCE (2). The first report of using silver nitrate in medicine in the CE was reported by Gabor in 702–705 for the treatment of wounds. Angelo Sal used silver nitrate in 1614 as a counterirritant, purgative, and for the treatment of brain infections (2). During

the same time, the Alchemists, who connected the seven planets to the seven days of the week as well as to parts of the body, connected silver to the moon and the brain, giving birth to terms such as “the silver moon” and ‘‘lunatic’’. Silver later came into vogue for the treatment of epilepsy when an epileptic stopped having seizures after he swallowed a large silver coin used to prevent him from biting his tongue (2). The solution of silver nitrate was commonly instilled within 1 hour after birth for prevention of gonococcal ophthalmia neonatorum (GON). Currently, it is argued that silver nitrate causes chemical conjunctivitis, pain and visual impairment. On the other hand, it is not considered as a perfect treatment to prevent or treat all cases of GON (3).

Silver nitrate is a potent oxidative chemical which extensively oxidize biochemical molecules, tissues and organs. Topical use of $AgNO_3$ resulted in topical oxidation of skin and change the skin color into brown. Inappropriate exposure to chemical compounds of silver would cause argyria (argyrosis), a condition which the skin turns blue or bluish-grey. The name is derived from the ancient Greek letter “argyros” which means silver. It may take the form of generalized argyria or local argyria. General argyria influences the entire body and more extensive areas of the skin, whereas local argyria influences confined areas such as patches of skin, parts of the mucous membrane or the conjunctiva (2).

Until the introduction of antibiotics in the early 1940s, silver colloids were the most common and powerful choice for antimicrobial therapy. Complexes of silver and proteins known as mild silver proteins were also employed. Topical, oral and systemic formulations of silver-based therapeutics were used. By 1940, silver-based medical products were commercially available in the United States in various forms such as solution, ointment, colloids or foils (4). Discovery of benzyl penicillin (Penicillin G) from the mould Penicillium notatum in 1928 by Alexander Fleming and its mass production was the endpoint for antimicrobial applications of silver compounds. But unfortunately it was not so long-lasting and resistant microbial strains strated to emerge. Now, antibiotics which used in the clinical setting beset by significant shortfalls, including weak antimicrobial activities, risk of microbial resistance, difficulty in monitoring and extending the antimicrobial functions, and difficulty in functioning in a dynamic environment.

This situation becomes more problematic when we deal with biofilms. It is well established that conventional antibiotics are not effective against biofilms. Biofilms are dense bio-structures with a cover of extracellular polysaccharide sheaths. As such, chemical molecules and antimicrobial agents have difficulty in penetrating the biofilm structure. Biofilm-associated bacteria are 100 to 1,000 times less-susceptible to antibiotics than planktonic bacteria, and agents active against planktonic bacteria, but not against biofilms, fail to cure patients(5). To overcome this resistance much higher than usual drug doses should be used, and high doses are often not tolerated by the host organism. In addition, the use of conventional antibiotics carries a major risk for resistance of viable bacteria. This issue becomes more complicated in situations where mixed bacterial biofilms are produced and multiple antibiotics are employed to target the complex microflora. Consequently, different measures of antimicrobial protection are required. Thus, effective and long-term antibacterial and biofilm-preventing materials constitute an immediate need in medicine and dentistry.

Reemergence of silver in novel form, silver nanoparticles

Over recent years, silver and its compounds have regained the attention of scientists in microbiology, medicine, biomedicine and nanotechnology fields. Nowadays, silver is used to control microbial life in a variety of medical applications, including dental work, catheters, and the healing of burn wounds (6-8), but not in the traditional form. Nanotechnology provides novel essence in all scientific and technological fields (9-18). Antimicrobial field was not an exception and so a modern form of silver (silver nanoparticles) appeared as the most common and applicable form of silver and an antimicrobial agent backed up by an ancient history. Nanotechnology provides a sound platform for adjusting the physicochemical properties of numerous materials to generate effective antimicrobials(19). Silver nanoparticles (AgNPs) may be strategically advantageous as active antibacterial agent since their surface area is exceedingly large relative to their size. Such properties increase the potency of AgNPs to the extents near the Ag+ ions without previously mentioned side effects of silver ions and silver nitrate. Consequently, AgNPs have been proposed to serve as alternatives to antibiotics to control bacterial infections.

The majority of antibiotics currently in use, generally affect three bacterial targets including cell-wall synthesis, translational machinery, and DNA replication (20). Unfortunately, bacterial

resistance may develop against each one of these modes of action. Mechanisms of resistance include enzymes which modify or degrade the antibiotic such as lactamases and aminoglycosides, modification of cell components such as cell-wall as seen in vancomycin resistance and ribosomes in tetracyclines resistance, and finally efflux pumps that provide multidrug resistance against numerous antibiotics (20). To prevent mutant strains development, some chemotherapy protocols suggest the use of various groups of antibiotics at a time. These protocols are based on the targeting multi-sites in the bacterial cells at a time. This is just what AgNPs, and more precisely, silver ion has done. Silver ions can affect almost all biomolecules in the bacterial cell. On the other hand, since AgNPs mode of action is mainly by direct contact with the bacterial cell-wall, without the need to penetrate the cells, most of the resistance mechanisms seen with antibiotics became irrelevant (21). Today, AgNPs are being increasingly reported to be an efficient antimicrobial agent against various pathogens in vitro and in vivo (9,22). The followings are some well-discussed mechanisms for the antimicrobial action of Ag+ ion and AgNPs which make silver an interesting tool against microbial infections.

Antimicrobial mechanisms for silver ions

- Effects on ions exchange

Silver cations inhibit phosphate uptake and exchange in microbial cells and cause efflux of accumulated phosphate as well as of mannitol, succinate, glutamine, and proline (23). Silver ions cause the release of potassium ions from bacteria; thus, the bacterial plasma or cytoplasmic membrane, which is associated with many enzymes, becomes an important target site for silver ions (24,25). Silver ion shave the ability to collapse microbial proton motive force. Considering the importance of the transmembrane proton gradient in microbial metabolism, it seems that the elimination of proton motive force should inevitably result in cell-death (26).

-Complex formation with DNA

Silver ions make complexes with bases present in DNA molecules and could potentially inhibit microbial DNA associated enzymes such as DNases (27).

- Enzyme inactivation

Ag+ ions can form complexes with electron donor functional groups on the amino acid residues such as thiol, carboxylate, phosphate, hydroxyl, amino, imidazole and indole leading to enzyme inactivation. While the main interactions were with thiol (sulfhydryl) groups(6,8,28,29), other target sites remain a possibility(3). Amino acids, such as cysteine, and other compounds containing thiol groups, such as sodium thioglycolate, neutralized the activity of silver against bacteria(30). In contrast, disulfide bond-containing amino acids, non-sulfur-containing amino acids, and sulfur-containing compounds, such as cystathione, cysteic acid, L-methionine, taurine, sodium bisulfate, and sodium thiosulfate are all unable to neutralize the activity of silver ions. Such findings declare that the interaction of silver ions with thiol groups in enzymes and proteins plays an important role in its antimicrobial action, although other cellular components, like hydrogen bonding, may also be involved (29). Silver was also proposed to act through binding to key functional groups of enzymes. Silver ions can also displace metal cationic cofactors from their actual binding sites in various enzymes (27).

-Inhibition of enzymatic oxidations

Silver ions might inhibit oxidation of glucose, glycerol, fumarate, and succinate in microbial metabolism pathway(27).

-Shrinkage of cytoplasm membrane

After silver ion treatment, the cytoplasm membrane would shrink and become separated from the cell wall. Cellular contents are then released from the cell-wall, and the cell-wall is degraded.

These phenomena suggest the possible antibacterial mechanisms by which silver ions inhibit bacterial growth, as well as cellular responses of both the gram-positive and gram negative bacteria to the silver ion treatment. Although the antibacterial mechanisms of silver is still not fully understood, several investigations have shown that the interaction between silver and the bacterial membrane compartments caused structural membrane changes and intracellular metabolic activity which may potentially lead to cell death (24,31,32).

- Making bacteria viable but non-culturable (VBNC)

The electrically generated silver ion solution exerts its antibacterial effect by making the bacteria viable but non-culturable (VBNC), in which the bacterial cells are in a state of very low metabolic activity and do not divide, while alive. In this state mechanisms required for the uptake and utilization of substrates leading to cell division were disrupted at the initial stage and caused the cells to undergo morphological changes and became smaller. These cells demonstrate reduced rate for nutrient transport, respiration, and synthesis of macromolecules. Sometimes, VBNC bacteria can remain in this state for over a year but finally die at the later stage (24).

- Deposition in vacuoles and cell-wall

Silver ions can deposit in the vacuoles and cell - wall as granules. They inhibit cell division and cause damages in the cell envelope and bacterial cell contents. In this condition, elongated cells are appeared and structural abnormalities would occur in the inner and outer membranes(24). The silver ion solutions showed better activity against gram-negative bacteria than gram-positive ones. This was possibly due to the thickness of the peptidoglycan layer, which may prevent the action of the silver ions through the bacterial cell wall (1, 24,32,33).

Antimicrobial mechanisms for silver nanoparticles (AgNPs)

The mode of action of AgNPs on the bacteria is not well known, while its possible mechanism of action might be related to the morphological and structural changes in the bacterial cells (34). The AgNPs show more efficient antimicrobial properties compared with other substances due to their extremely large surface area, which provides better contact with microorganisms (34). Xiuet al. have shown that antimicrobial action of AgNPs is completely oxygen dependent and AgNPs are inefficient in the absence of oxygen. This effect is due to oxidation of AgNPs in the presence of oxygen and therefore release of Ag+ ions from the AgNPs. They suggest that that Ag+ ion is the definitive molecular toxicant and ruled out direct particle-specific biological effects by showing the lack of toxicity of AgNPs when synthesized and tested under strictly anaerobic conditions that preclude Ag oxidation and Ag+ release.

However, many environmental factors affect the AgNPs lethal potency to microorganisms including, pH, temperature and aeration. The physicochemical properties such as size, shape and chemical modification would greatly affect antibacterial activity of NMs(21,35). Xiuet al. suggested that AgNPs morphological properties known to affect antimicrobial activity are indirect effectors that primarily influence Ag+ ions release. Accordingly, antibacterial activity could be controlled (and environmental impacts could be mitigated) by modulating Ag+ release, possibly through manipulation of oxygen availability, particle size, shape and type of coating(36,37). On the other hand, it has been shown that within a period of 24 h, less than 5 μ M of free silver ions are released into solution from AgNPs. Therefore, the antimicrobial effect of AgNPs cannot be solely related to the release of Ag+ ions from the nanoparticles(1).

- Production of reactive oxygen species (ROS)

Released Ag+ ions from the AgNPs catalyse production of reactive oxygen species (ROS) in the Fenton's reaction (10,15). ROS, considered the most effective determinant for both in-vitro and in-vivo cytotoxicity of NMs, are induced indirectly due to respiratory chain disruption or directly by the AgNPs themselves (38). Severe oxidative stress can cause a burst of ROS leading to damage to all the cell's macromolecules such as lipid peroxidation, alteration of proteins, inhibition of enzymes, and RNA and DNA damage. ROS can cause cell death at high concentrations while low doses would cause severe DNA damage and mutations. In some cases, where the production of ROS is induced by visible or UV light the toxicity is photocatalytic. For instance, TiO2 nanoparticles were shown to induce lipid peroxidation under near-UV light, which leads to respiratory dysfunction and death of E. coli cells.

Some other nanoparticles effects include direct inhibition of specific essential enzymes, induction of nitrogen reactive species (NRS) (39), and induction of programmed cell-death(21).

- Impacts on the cell membrane

Membrane damage occurs when AgNPs bind to the bacterial cell-wall and membranes, leading to membrane depolarization, and loss of integrity which leads to imbalance of transport, impaired respiration, interruption of energy transduction and eventually cell lyses and death(40). It is well known that gram-negative bacteria possess an outer membrane outside the peptidoglycan layer lacking in gram-positive organisms. The essential function of the outer membrane is to serve as a selective

permeability barrier, protecting bacteria from harmful agents, such as detergents, drugs, toxins, and degradative enzymes, and penetrating nutrients to sustain bacterial growth. The structure and chemical composition of the outer membrane in E. coli cells has been widely studied. The lipid bilayer of outer membrane is asymmetric whereby the inner layer mostly contains close-packed phospholipid chains, while the outer layer is composed of lipopolysaccharide (LPS) molecules. It has been estimated that approximately 3.5 million molecules of LPS cover three quarters of the surface of E. coli, while the remaining quarter composed of membrane proteins. Experimental evidences have proved that the LPS layer of the outer membrane plays a key role in providing a selective permeability barrier for E. coli and other gram-negative bacteria. Evidences also showed that mutant altered LPS structures could increase permeability compared with that of native cells (41). The AgNPs might enhance the permeability of membrane to reduce sugars and proteins in the cells. So it could be conferred that disturbance of membranous permeability would be directly related to bacterial growth inhibition. But it is not yet clear where the damage takes place, on the lipopolysaccharide or membrane proteins in outer membrane (42).

TEM images of membrane vesicles (MVs) showed that the MVs were dispersed when treated with AgNPs, and the membrane components became disorganized and scattered from their original order. The TEM micrographs of E. coli cells treated with AgNPs showed big gaps in the cell membrane, and the bacteria were almost disorganized to several parts. SEM micrographs of E. coli cells treated with AgNPs showed many fragmentary bacteria. These events suggest possible antibacterial mechanisms by which AgNPs inhibit bacterial growth, as well as cellular responses to the AgNPs treatment.

So, the antibacterial action of AgNPs may be first described as increasing the permeability of outer membrane, resulting in the leakage of cellular contents. Secondly, nanoparticles enter the inner membrane and inactivate respiratory chain dehydrogenases. In fact, when AgNPs enter the bacterial cells, they form a low molecular weight region inside the bacteria. Thus, the bacteria form conglomerates to protect the DNA. Consequently, the nanoparticles preferably attack the respiratory chain. Disruption in cell division would finally lead to cell death(34). A little exposure of AgNPs to bacterial cells would result in the accumulation of envelope protein precursors. It can be proposed that AgNPs may target bacterial membrane, leading to a destruction of proton motive force(1). AgNPs could induce collapse of membrane by affecting some proteins and phosphate lipids, resulting in cell disintegration and eventually cell-death (42).

Resistance to silver compounds

Resistance to silver compounds is related to plasmids and genes. Salmonella plasmid pMGH100 involves nine genes in three transcription units that make this bacteria resistant to silver (43). Silver compounds significantly reduce the cellular chemiosmotic potential(44,45). According to previous studies, sensitivity to silver may differ from bacterial strains or when different culture media are used (46).

Synthesis of AgNPs

Several chemical and physicochemical techniques have been introduced for the production of AgNPs with various characteristics. In the almost all of these techniques the bottom-up methods were applied, in which the silver ion was used as silver precursor. By reduction of Ag+ ions to Ag0 and agglomeration of silver atoms gradually, AgNPs are made. Finally prepared particles were coated by hydrophilic and biocompatible coatings to make them physically and chemically stable (47-53). However, these methods suffer from high energy consumption, use of toxic chemicals and organic solvents which are potentially dangerous to environment and human health.

The progressive applications of AgNPs in medicine, science and technology lead to an increased demand for AgNPs production in sustainable proses. Therefore, attempts were made to use green chemistry for synthesis AgNPs via environment-friendly manners. By now, various approaches have been developed in this way and AgNPs are synthesized by using microbial secretory compounds or plant extracts. Biochemical compound have the ability for simultaneously reduction and capping AgNPs without using any toxic chemical or organic solvent (54-77). Oxygen-bearing functional groups were identified as active groups for entrapment and reduction of Ag+ ions by biologic compounds(9).

Toxicity to human cells and tissues

Despite several antibacterial advantages of NMs, they also have some significant shortcomings. In fact the subsequent oxidative stress, disturbing enzymes activity, membrane and DNA damage, make human cells and tissues susceptible to AgNPs. Compared with other metals, silver exhibits

higher toxicity to microorganisms while it exhibits lower toxicity to mammalian cell (1).

Despite mentioned disadvantages, recent studies show that AgNPs are still efficient as potential antibacterial agents (21).

Conclusion

Silver and silver-based compounds are among the most potent antimicrobial agents with ancient history of medical applications. Traditionally, silver ions and silver ion complexes were used as effective compound against microorganisms, such as silver nitrate which had considerable side effects. Nowadays, AgNPs are using as a novel and potent formulation of a traditional antimicrobial agent. AgNPs attack multiple sites on the microbial cells and this is what targeted by multidrug therapy to reduce the possibility of the emerging resistance strains. Various mechanisms have been known for the effect of Ag+ ions and AgNPs on the microbial cells. However, much further research is needed to identify the exact impact of AgNPs on animal, plant and human cells.

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