Elsevier Public Health Emergency Collection

Public Health Emergency COVID-19 Initiative

Biochem Biophys Res Commun. 2020 Nov 26; 533(1): 195–200.

Published online 2020 Sep 11. doi: 10.1016/j.bbrc.2020.09.018

PMCID: PMC7486059

PMID: 32958250

Potent antiviral effect of silver nanoparticles on SARS-CoV-2

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Associated Data

Supplementary Materials

Abstract

1. Introduction

The elemental metal, Silver (Ag) has broad spectrum antimicrobial action against various bacteria, fungi and viruses. Due to their versatility, Ag nanoparticles (AgNP) have currently found their way as a microbicide for biological surfaces in various forms such as wound dressings, medical devices, deodorant sprays and fabrics. Several studies have demonstrated the potent antiviral action of AgNPs against various human pathogenic viruses such as Respiratory syncytial virus (RSV), Influenza virus, Norovirus, Hepatitis B virus (HBV) and Human immunodeficiency virus (HIV) [1]. In addition to these viruses, since Ag has been demonstrated to kill SARS-CoV, we hypothesized the strong possibility of AgNPs to inhibit SARS-CoV-2 [2,3]. Till date there are no studies directly demonstrating the effect of AgNPs on SARS-CoV-2. We tested colloidal silver (cAg), plain elemental Ag nanoparticles of different diameters ($AgNP_n$) and polyvinylpyrrolidone capped 10 nm silver nanoparticles ($PVP–AgNP_{10}$) against SARS-CoV-2 to find the most effective size and concentration of Ag that could inhibit SARS-CoV-2. We propose that AgNPs could be used on inanimate and non-biological surfaces to efficiently control the ongoing COVID-19 pandemic while simultaneously exercising care not to abuse it.

Note Silver (Ag) = Normal Silver (Colloidal Silver)
Silver (AgNP) = Nano Particle Silver (ABL has the only patented Nano Silver in the World)

5. Discussion

Ag is long known for its antimicrobial effect and the antiviral property of AgNPs is being extensively researched with renewed interest in the recent past [1]. The exact mechanism by which AgNPs exert its killing effect on viruses is still obscure. However, it has been consistently observed that AgNPs interact with the structural proteins on the surface of extracellular viruses to inhibit infection in the early phase, by either preventing viral attachment or entry, or by damaging the surface proteins to affect the structural integrity of virions [11,12]. In the current study, we have obtained similar findings in the VPrA where AgNPs effectively inhibits extracellular SARS-CoV-2 to protect the target cells from infection and the pseudovirus entry assay revealed that AgNPs interfere with viral entry.

AgNPs have been shown to preferentially bind to viral surface proteins rich in sulfhydryl groups and cleave the disulfide bonds to destabilize the protein, thereby affecting viral infectivity [11,13]. Studies on HIV have shown that AgNPs associate to the disulfide bonds that are in close proximity to the CD4 binding domain of the gp120 surface protein [11]. Hati and Bhattacharyya have demonstrated the importance disulfide bonds in binding of SARS-CoV-2 spike protein with the angiotensin converting enzyme-2 (ACE2) receptor and the disruption of which lead to impaired viral binding to the receptor [14]. Considering the mechanism of action of AgNPs shown by other authors, it can be presumed that AgNPs exert their antiviral effect on SARS-CoV-2 by disrupting the disulfide bonds on the spike protein and ACE2 receptors. Further studies are being conducted to find the antiviral mechanism of AgNPs on SARS-CoV-2 and elucidate it in detail subsequently.

AgNPs have also been claimed to possess intracellular antiviral action by interacting with viral nucleic acids [15]. We observed a partial antiviral effect in CPrA, as there was some amount of reduction in the viral load in cells pre-treated with PVP-$AgNP_{10}$. While the reason for this effect is not known at present, it is possibly explained to be either due to the destruction of disulfide bridges on ACE2 receptor or due to a true intracellular mechanism (there by inhibiting serial viral infection of newly produced virus from infected cells to uninfected cells). Also, since Ag binds non-specifically to proteins, their use as antiviral agents might also cause some cellular dysfunction. Further studies are required to more precisely explain the holistic effect of Ag *in vivo*.

Several studies have reiterated the size dependent antiviral effect of AgNPs with particles around 10 nm diameter being most effective [1]. This has been attributed the higher stability of interaction to the viral protein achieved by 10 nm particles which is not capable by larger particles [11]. Consistent with this, we also observed *anti*-SARS-CoV-2 activity only with AgNPs of diameters ranging from 2 to 15 nm. Our immunofluorescence study corroborated the above phenomenon, as we observed that PVP-$AgNP_{10}$ completely inhibited SARS-CoV-2 but $AgNP_{100}$ did not.

AgNPs can be generated by several methods and can contain reducing agents and capping agents along with the metal particles [16]. Coated or capped AgNPs are found to be more advantageous than plain AgNPs as coating increases stability, decreases agglomeration and reduces cytotoxicity of AgNPs [17]. Among the coated AgNPs, PVP capped nanoparticles are widely studied for biological use. It has been observed that PVP coating of AgNPs does not

hinder their antiviral activity while other coating agents do [18]. PVP-$AgNP_{10}$ has been demonstrated to possess excellent antiviral activity against enveloped viruses such as RSV and HIV [11,19]. This was the rationale to select PVP-$AgNP_{10}$ for the study and we have demonstrated the robust antiviral effect of PVP-$AgNP_{10}$ against SARS-CoV-2.

Antiviral effect of AgNPs is also concentration dependent. Most studies have observed the antiviral efficacy of AgNPs at concentrations ranging between 10 and 100 ppm [1]. However, 0.5 ppm cAg has been shown effective in inhibiting Influenza virus and is the least concentration that has been reported to show antiviral activity [20]. In the current study, we observed naked AgNPs to inhibit SARS-CoV-2 at concentrations ranging between 1 and 10 ppm and become cytotoxic to mammalian cells from 20 ppm and above.

Cytotoxicity of AgNPs to mammalian cells depends on the cell type and also the type of AgNPs. Mehrbod et al. have observed cytotoxicity in Madin-Darby Canine Kidney (MDCK) cells with naked cAg particles at concentrations higher than 0.5 ppm [20]. Naked AgNPs with NaBH4 reducing agent were found to induce apoptosis in colon adenocarcinoma cells at 11 ppm, while Citrate-stabilized naked AgNPs have been observed to exhibit cytotoxicity at concentrations higher than 30 ppm [21,22]. In this regard, PVP coated AgNPs have been demonstrated to be the least cytotoxic with no demonstrable cytotoxicity even at 50 ppm in human alveolar basal epithelial cells [19]. Smaller particles have a higher toxic potential due to the greater surface area of interaction with the bound protein [23]. We observed this effect as $AgNP_2$ showed cytotoxicity even at 2 ppm while none of the bigger particles were cytotoxic at this concentration. Therefore, care should be exercised when Ag is used on biological surfaces.

Various ingestible and inhalable formulations of Ag are being marketed as cure for COVID-19, which available to purchase over the counter. The cytotoxic potential of these formulations should be considered before personal use. Also, Ag is a very broad spectrum microbicide. Illicit use of Ag might create an imbalance in the commensal microbiota leading to unforeseen consequences [24]. AgNPs can be used on a variety of inanimate surfaces to combat the ongoing COVID-19 pandemic [3]. Ag coated masks have been found to be effective in inhibiting SARS-CoV-2 and could potentially be effective when applied on the air filters of air conditioners and medical devices [25]. AgNP incorporated polycotton fabrics have been proven to inhibit SARS-CoV-2 [26]. Ag based sanitizers and disinfectants are also being used for disinfection of hands and inanimate surfaces respectively [27]. However, the effect of AgNPs on influencing the microbial life when released in the environment is unknown [16]. A proper disposal protocol should be developed for Ag containing products to avoid causing untoward imbalances in the environmental microbial pattern when discarded after use.