Preventing Viral Infection with Silver Nanoparticles

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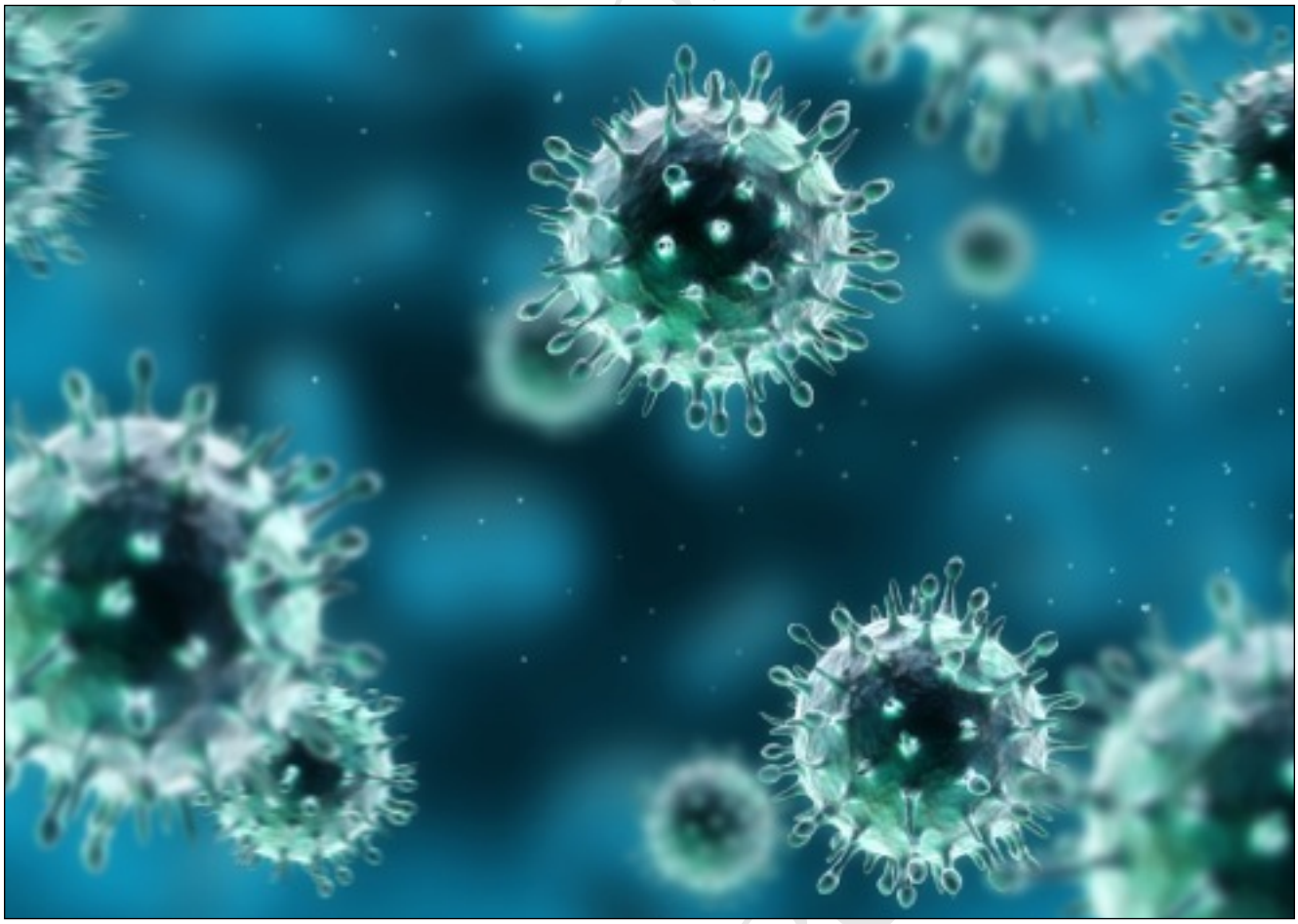
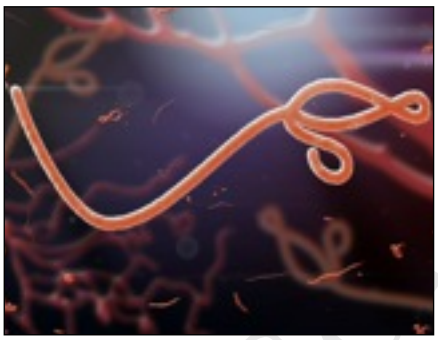
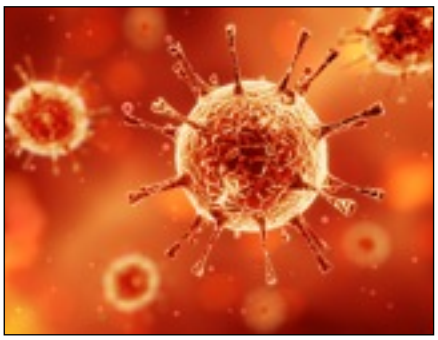


Introduction

Viruses form the most substantial causes of disease and death in the world (1). They cause some of the most miserable diseases that include disfigurement, disability, paralysis, cell mutation and hemorrhagic fevers on their way to a torturous death (1). They are found in the air, water, soil and in all forms of life (1). They are often contagious and sometimes dormant waiting for a time where there is less immunity against their genetic makeup.

Viruses in general are made up of incomplete segments of DNA looking to find a binding site made up of healthy DNA. When the virus binds to the healthy DNA it causes a mutation or change in the sequence of that DNA and uses this reproductive ability against its host. So to be infected by a virus is truly genetic attack at the sub-cellular level. When antibodies are produced, effectively controlling the virus, it is then that the virus uses its incomplete genetic material to recombine with other viruses and becomes a new genetic structure immune to the antibodies designed to keep it from erupting; and a new disease becomes epidemic.





Virus effects are so detrimental that they can cause long term suffering from persistent diseases that can lead to immunodeficiency, cancer, and are some of the most contagious diseases, infecting and killing as much as 40% of the population in one area. However, for today's most pressing viral pathogens, there is still no vaccine available (CDC). A large number of viruses are prevalent today such as HIV, Rhinovirus, Hepatitis C, HPV, influenza, and Ebolavirus, where there is no vaccine available (WHO). An increasing number of attempts are being made to develop vaccines for such deadly diseases, without complete success.

Viruses are the most difficult infectious diseases to treat with modern medicine because the virus is neither alive nor dead. They consist of a nuclear capsid (microscopic cellular container) that contains short incomplete sections of genetic material (DNA or RNA). The virus binds to a healthy cell surface, then fusion between the cell and virus occurs, after which the virus injects these incomplete segments of DNA or RNA into the cell where they bind with normal DNA/RNA and interfere with the normal replication of the cell. This interference causes abnormal function from the cell. In effect, the infecting virus changes the function of the cell by sabotaging the genetic material and does so in a way that the immune cells cannot detect or destroy the virus. This is because the virus is inside a healthy cell. The virus interferes with the healthy cells genetic material triggering abnormal growth by

reproducing abnormal virus clones. These are like a Trojan horse that gains access to preferred areas yet is filled with tens of thousands of offspring hiding inside the normal cell waiting to be released so they can infect the nearest healthy cell. When there are tens of thousands of replicated virus daughters inside the cell, it bursts open releasing the newly formed viruses, and the body is attacked by millions of viral offspring. These newly released viruses attach to healthy cells, fuse with it, inject the incomplete genetic material into the cell and begin the replication/infection process all over again.

There are several ways to destroy, prevent or build immunity against a virus; The most common way is to be vaccinated or exposure to the virus, where your body will build immune cells precisely against that virus. If the body receives a vaccine it constructs immune cells that identify all future exposures to that specific virus and initiates an immune response that destroys, engulfs, and eliminates that virus now and in the future. These are called memory cells and antibodies. They take about two weeks to develop and provide immunity from that viral disease for the rest of your life. There are some drugs that stop the replication of the virus so that its effects slowly dissipate. Some drugs boost immune cell numbers and function in a way that the immune system can engulf and eliminate the virus. There is one anti-viral drug treatment costing over $50,000 US dollars and it is only one of the three drugs prescribed for hepatitis C infection (37).

Another way to prevent viral infection is to somehow prevent the virus from attaching to healthy cells. If the virus cannot attach to the healthy cell it cannot do anymore damage. There are substances that block this viral cell attachment. This is true prevention because the virus is never allowed into the healthy cells and all the healthy genetic material is protected.

Prevention from viral attack is far superior to trying to treat the virus after infection because there is so much DNA that has been altered. Some medical professionals refer to vaccination as prevention but this is not totally true due to the fact that the patient may get the viral disease they are being inoculated against or there could be an abnormal immune response leading to persistent autoimmune syndromes like arthritis, inflammatory bowel, lupus, MS, paralysis or even death. In addition the virus may mutate and become a different virus rendering the vaccination unable to provide protection from disease. Since we cannot eliminate all viruses in all living organisms, the better preventive choice is to prevent the binding of the virus to the healthy cell. The most common non-prescriptive way to prevent viral attachment is with the use of silver liquid and gel.

Silver has thousands of scientific studies documenting its anti-viral benefits. The following is a review of the Scientific Publications documenting the antiviral benefits of liquid silver including Ebola virus.

Ebola Virus

Ebola virus is one of the four ebolaviruses known to cause disease in humans. It has the highest case fatality rate of these ebolaviruses, averaging 83% since first described in 1976, (Yamboku,1976), although fatality rates up to 90% have been recorded in one epidemic (2002–03) (1).

There is currently no cure for the Ebola virus (WHO, CDC). It is extremely contagious and the CDC has classified it as an extreme emergency. It exists predominantly in Africa where it has killed thousands of people. It is transmitted through direct contact with infected body fluids where it causes diarrhea, vomiting, internal and external bleeding, dark bloody feces, macopapular rash, blood shot or bleeding eyes due to hemorrhage of arterioles through out the body. This virus directly affects the blood platelets and disrupts the clotting mechanisms resulting in bleeding throughout the internal and external body, and has been reported to be 70 - 90% fatal (1). Because it takes up to 21 days for the symptoms to appear, it may be possible for people to be contagious for weeks spreading body fluids like mucus, saliva, blood, semen, sweat tears that could be infecting people and they don't know it for three weeks (CDC). You could actually be infected now and not know about it until the fever and rash appear 21 days later (CDC).

The only true and effective treatment is prevention. Although there are reports about a serum, it is not available to the masses nor is it approved as a treatment yet. The Centers for Disease Control recommend the following three methods of treatment:

1. Balance the patients fluids and electrolytes. This is due to the massive loss of blood from hemorrhaging and water loss due to diarrhea and fever over 101.5 degrees.
2. Maintain normal blood pressure and oxygen status.
3. Treating them for any complicating infections.

Because the Ebola virus systematically dismantles the immune system where the patient becomes immune compromised before the fever even appears. The secondary infections like strep, staph, pseudomonas or other pathogens can cause death and or weaken the immune system and they can easily be treated or prevented with the daily use of a structured silver water.

It is my recommendation that all concerned individuals swallow two teaspoons of liquid structured silver (preferably alkaline) twice a day to help prevent the secondary infections and potentially block the attachment of the virus to healthy cells. This opinion is based on the published mechanisms of action that demonstrate how silver functions and could ultimately be the preventive agent for Ebola and other viruses.

Silver liquid (AG4O6, AG4O4, AG 2O4) and gel destroy bacteria, viruses, some parasites and fungi through the individual and or combination of mechanisms of action including; (A) Selective binding of pathogens, (B) Stealing or firing an electron from or at pathogens (C) Recurring cyclical Redox reaction (chemical), (D) Viral replication interference, (E) Resonant frequency that results in the destruction of pathogens (as a multi-phasic, di-electro-magnetic semi-conductor producing a resonant frequency that destroys disease causing pathogens), (F) Nano-electrocution, (G) Modulation of the immune system (stem cells) producing healing benefits, (H) when using a pH balanced silver; alkaline induced pathogenisis, (I) binding to glycoprotein cell surfaces thus preventing viral entrance into healthy cells. (J) Silver binds to Nitrogen and oxygen preferentially, thus destroys pathogens producing these metabolically.

Due to the fact that there are sufficient scientific claims for the use of silver, I would do the following in order to prevent an Ebola virus illness:

- Drink a liquid silver nanoparticles two teaspoons twice a day for prevention.
- If I'm exposed to possible sick people I would double that to 4 teaspoons twice daily.
- If I believe I have been infected I would drink 2 tablespoons twice a day.
- I would stay hydrated.
- I would get 8 hours sleep a night in a room with a HEPA filter to filter out viruses.
- I would apply silver gel to any rash for any reason twice a day.
- I would wear protective clothing and avoid any body fluid transfer.

Mechanism of Action

Silver can bind with the glycoproteins on the surface of the healthy cells and block or prevent the Ebola virus from entering the healthy cell thus protecting and preventing viral disease from origination.

Ebola expresses a class I fusion glycoprotein that is highly *N-* and *O-glycosylated* and acylated at its cytoplasmic tail. To prevent Ebola from binding to a healthy cell silver can bind to the Nitrogen and Oxygen glycosolated proteins and competitively inhibit any further viral

activity thus effectively preventing viral infection. The tiny silver particles (5-10 nm) are the correct size to fit into the molecular structure needed for competitive inhibition of the Ebolavirus.

There are two candidates for host cell entry proteins. The first is the host-encoded Niemann–Pick C1 (NPC1), a cholesterol transporter protein, appears to be essential for entry of Ebola virions into the host cell, and for its ultimate replication (13,14). In one study, mice that were heterozygous for NPC1 were shown to be protected from lethal challenge with mouse-adapted Ebola virus. (13). In another study, small molecules were shown to inhibit Ebola virus infection by preventing viral envelope glycoprotein (GP) from binding to NPC1 (14,15). Hence, NPC1 was shown to be critical to entry of this Filovirus, because it mediates infection by binding directly to viral GP (14).

When cells from Niemann Pick Type C patients, who are lacking this transporter, were exposed to Ebola virus in the laboratory, the cells survived and appeared impervious to the virus, further indicating that Ebola relies on NPC1 to enter cells(13). Mutations in the NPC1 gene in humans were conjectured as a possible mode to make some individuals resistant to this deadly viral disease (14). The same studies described similar results regarding NPC1's role in virus entry for Margurg virus, a related Filovirus. A further study has also presented evidence that NPC1 is critical receptor mediating Ebola infection via its direct binding to the viral GP, and that it is the second "lysosomal" domain of NPC1 that mediates this binding (16).

In one of the original studies, a small molecule such as silver nanoparticle, was shown to inhibit Ebola virus infection by preventing the virus glycoprotein from binding to NPC1 (13,14). In the other study, mice that were heterozygous for NPC1 were shown to be protected from lethal challenge with mouse adapted Ebola virus (15). Together, these studies suggest NPC1 may be potential therapeutic target for an Ebola anti-viral drug.

Silver nanoparticles of the size 5-10 nm should fit and attach to the glycoproteins on the surface of cells which will inhibit viral attachment, resulting in the prevention of viral entry, viral replication, and viral infection. The Ebola virus enters the same cell site as influenza. Dr Pedersen (2010), demonstrated prevention of influenza using silver nano particles so it is probable that Ebola could be prevented with the same silver pre-treatment as is successful in influenza.

This is because the individual glycoproteins (GP) on the surface of healthy cells have a molecular structure with spacing of about 10 nm. Silver nano particles have been shown to be of this exact size and proven to bind with this type of glycoprotein's size, chemical

structure, and charge. In fact, silver seeks the nitrogen that makes up the glycoprotein coat. In addition, other viruses with similar glycoprotein surface structures are totally inhibited by silver nanoparticles. It is my suggestion that this will be the same type of viral inhibition in the Ebolavirus.

Anti-bacterial and anti-fungal properties of silver nanoparticles (AgNPs) have been widely studied (27,28,29). Recently, antiviral properties of AgNPs have been reported during in vitro studies with HIV-1 (30,31), HBV (32) and influenza virus (33). Lara et al. (2010) showed that AgNPs can bind to one of the HIV surface glycoprotein (gp 120) and inhibit virus-to-cell attachment (31). Baram-Pinto et al. (2009) used mercaptoethane sulfonate capped AgNPs to inhibit HSV-1 attachment to cell host membrane and thus infection (34).

Silver nanoparticles have exhibited statistically significant antiviral activity at doses as low as 2 μg mL-1 ($p = 0.005$). The minimum inhibitory concentration 50% (IC50) of AgNPs against the virus was measured at 16 μg mL-

A recent report suggests that inhibition of HIV-1 by AgNPs occurs at an early stage of infection, prior to assembly of new particles in the cell (40). Targeting the early step of viral infection is promising approach because the site of the action of inhibitor is extracellular and accessible, but it is very difficult to target early steps of virus attachment (1).

Silver is static in its metallic state but reacts with the moisture in the skin and the fluid of the wound and gets ionized. The ionized silver is extremely reactive, as it binds to tissue proteins and brings structural changes in the cell wall of bacteria and nuclear membrane leading to cell distortion and cell death. Silver binds to microbial genome (DNA or RNA) by denaturing and inhibits its replication (14). Silver vessel are also used to make water potable which becomes sterile. As the concentration of Ag+ ion is very low, this has been called oligodynamic action (1).

Mechanism of Silver in the Cell

The mechanism of action of silver in the cell is associated with its interaction with thiol group which is found as a functional group in the respiratory enzymes of bacterial cells. Silver gets attached to the cell wall and membrane and then it inhibits the respiration process (17). In the case of E. coli, silver acts by inhibiting the uptake of phosphate and releasing carbohydrates like mannitol, succinate, and amino acids like proline and glutamine and phosphate from E. coli cells (18).

In this era, when drug and vaccine development for the removal of various viral diseases is riding high, some viral strains have emerged that are resistant against the drugs and vaccines, like HIV (1). So it is important to introduce the multidisciplinary approaches with the classical epidemiology, along with the clinical phases to introduce a new drug or vaccine which proves highly beneficial against the resistant strain. Nanotechnology is the one that gives the opportunity to re-discover biological properties of ancient antimicrobial and antiviral compounds. Nanoparticles, mainly silver have antiviral activities against the many viruses of today that are playing havoc with lives worldwide. Extensive research and clinical trials need to be carried out so as to accentuate the efficacy of this medical marvel towards betterment of the health of the global population (1).

Silver nanoparticles are mainly used for the antiviral activity against viruses like HIV-1 (42, 43, 44, 45), hepatitis B virus (46), monkey pox virus (8), Tacaribe virus (47), influenza virus (48), herpes simplex virus (49) and respiratory syncytial virus (50). For inhibition of viruses, nanoparticles of size ranging from 1-100 nm are mostly used (41). The size of the nanoparticles has a major role in the interaction; smaller the size more the interaction and more inhibition takes place. In addition nanoparticles come into the cell and apply their size dependent phenomenon which cause antiviral activity with their viral genome (DNA or RNA) (41). Smaller sized nanoparticles enter into the host cell and then enter in the viral genome where they block the cellular factors and/or the viral vectors which help in the viral replication. Alternatively, they may get attached to viral genome so that no polymerase action takes place and no further formation of progeny virions takes place.

Nanoparticles are located at a particular location, having regular spatial correlation observed among three particles. The practical spatial arrangements show a relationship of the location of gp 120 glycoprotein knobs in the structural model for HIV-1 (42).

Regular spatial arrangements are determined by the HAADF (High Angle Annular Dark Field) scanning transmission electron microscopy. In the case of HIV-1 Virus, viral envelope mainly consists of a densely-packed lipid membrane. For the glycoprotein knobs, a localized region of lower density is observed due to the occurrence of membrane-spanning gp 41 glycoprotein. Therefore, glycoprotein areas appear darker than the remaining viral envelope. The centre to centre spacing is approximately 22 nm in glycoprotein knobs and spacing between silver nanoparticles is approximately 28 nm which is associated with the expected spacing between glycoprotein knobs. The experiential spatial arrangement of nanoparticles, the gap between nanoparticles and the uncovered sulfur-bearing remainder of the glycoprotein knobs are the striking sites for nanoparticles interaction suggesting that

silver nanoparticles interact with the HIV-1 virus by means of better binding to the gp 120 glycoprotein knobs. The nanoparticles interact with the glycoprotein knob having size mainly 1-10 nm, sometimes 14 nm. Greater than this are not able to attach to the virus envelope (42).

Other Useful Applications

It is seen that silver nanoparticles have the antiviral and antimicrobial activities against several pathogens. Their utility is incorporated in materials and biomedical applications (53). Nanoparticles are used as additives in health care such as bandages and catheters for healing of wounds and burns in less time. Ag/Na carboxymethyl cotton burn dressing is used for the applications in surgical dressings. They are also used in common products such as water purification systems, domestic products, cosmetic products and emulsions to prevent harmful micro flora (54).

HIV-1

Silver nanoparticles have proven to exert antiviral activity against HIV-1 at non-cytotoxic concentrations (36). The anti-viral activity from silver nanoparticles occurs at an early stage inhibiting viral entry or as an anti-viral agent destroying the virus on contact. It was shown that the silver nanoparticles bind to a healthy cell surface glycoprotein (gp120) in w manner that prevents CD4-dependent virion binding fusion and infectivity, acting as an effective virucidal agent (36, 6).

These properties make silver nanoparticles a broad-spectrum agent not prone to inducing resistance that could be used preventively against a wide variety of circulating HIV-1 and other viruses (36).

H1N1 Influenza

Silver nanoparticles inhibited influenza H1N1 by preventing virus cell bonding. For all sizes of AgNPs tested, antiviral activity against H1N1 influenza A virus increased as the concentration of AgNPs increased. Size dependence of the silver nano-particles on antiviral activity was also observed: antiviral activity was generally stronger with smaller silver nanoparticles (2). it is likely that the antiviral activity of silver nanoparticles against several other types of viruses is due to direct binding of the AgNPs to viral envelope glycoproteins, thereby inhibiting viral penetration into the host cell [19,8,24,26].

Silver nanoparticles (AgNPs) are well-known anti-microbial materials effective against many types of bacteria [17,18, 3] and fungi [4]. The anti-bacterial and anti-fungal activities of AgNPs are mainly due to the inhibition of respiratory enzymes by released Ag+ ions [17, 5]. Recently, the anti-microbial activities of Ag NPs against viruses such as HIV- 1 [19,7], hepatitis B [8], herpes simplex [9], respiratory syncytial [10], monkeypox [20], Tacaribe [12], and H1N1 influenza A virus [21,22] have also been investigated. Unlike its antibacterial and anti-fungal activities, the major antiviral mechanism of AgNPs is likely the physical inhibition of binding between the virus and host cell. A dependence of the size of AgNPs on antiviral activity was observed for the viruses mentioned above; for example, AgNPs smaller than 10 nm specifically inhibited infection by HIV-1 [19].

Previous studies showed that AgNPs have anti-viral activity against influenza A virus [21, 22]. It is likely that the anti-viral activity of AgNPs against several other types of viruses is due to direct binding of the AgNPs to viral envelope glycoproteins, thereby inhibiting viral penetration into the host cell [19,8,24,26].

Gold nanoparticles of different sizes, 2nm and 14 nm were used in which only 14 nm gold nanoparticles inactivate haemagglutination attachment in the nano molar range. They demonstrate that the activity depends on the particle size of the interacting ligand/receptor molecules. Therefore, it has been proven that Sialic-acid-functionalized gold nanoparticles are capable to successfully reduce viral infection (48).

The mechanism of receptor-mediated endocytosis brings the enclosed virus particle within the cytoplasm. Inside the late endosome, atmospheric acidification activates a conformational change of Haemagglutinin (HA), which mediates the protein fusion of the endosomal membrane with the viral covering ending with the discharge of the nucleoproteins and genome fragments into the cytoplasm (41, 48). This means that gold nanoparticles bond with the gatekeepers on the surface of the cell and prohibit the virus from attaching bonding and preventing any and all viral replication form infecting the healthy cell as long as there is the exact size of gold or silver nanoparticle blocking viral attachment.

The sizes of silver nanoparticles for the MPV inhibition are 10 nm, 25 nm and 80 nm. internalization of silver nanoparticles suggests that a possibility is there for the disruption of intracellular pathway that attenuates the viral replication (51).

Herpes Simplex

At 46 nm, silver nanoparticles produced 100% inhibition of the Herpes virus at the concentration of 2.5 μg/ml (35). This is significant because of the damage that herpes virus can cause on a long term basis. In addition, there is no cure for all herpes viruses. Silver nanoparticles used preventively can stop the virus from erupting if silver comes in contact with the virus during the first four hours of viral activation.

Hepatitis B

Silver nanoparticles inhibit hepatitis B virus replication (39). Silver nanoparticles could inhibit the in vitro production of HBV RNA and extracellular virions (39). It is hypothesized that the direct interaction between these nanoparticles and HBV double-stranded DNA or viral particles is responsible for their antiviral mechanism (39). Silver nanoparticles used in combination with interferon and other treatments show promise as combination therapy.

Tacaribe

Real time PCR, Confocal microscopy, and Transmission Electron Microscopy (TEM) shows the activity of silver nanoparticles against the Tacaribe virus. The Confocal microscopy and transmission electron microscopy show the internalization of TCRV into the Vero cells; 10nm silver nanoparticles were able to enter into the cells and also in the same endosome while the 25nm silver nanoparticles enter into the individual endosome.

Real time PCR shows the quantitative results which shows that silver nanoparticles inhibit the virus at the concentration of 25µg/ml and 50µg/ml all silver nanoparticles shows the significant reduction in the amount of S segment expression (47).

Exposing the tacaribe virus to silver nanoparticles prior to infection actually facilitated virus uptake into the host cells, but the silver-treated virus had a significant reduction in viral RNA production and progeny virus release, which indicates that silver nanoparticles are capable of inhibiting arenavirus infection in vitro (55). The inhibition of viral replication must occur during early replication, although pre-infection treatment with silver nanoparticles is very effective, the post-infection addition of silver nanoparticles is only effective if administered within the first 2-4 hours of virus replication (55).

Silver nanoparticles are capable of inhibiting a prototype arenavirus at non-toxic concentrations and effectively inhibit arenavirus replication when administered prior to viral

infection or early after initial virus exposure. This suggests that the mode of action of viral neutralization by silver nanoparticles occurs during the early phases of viral replication (55).

Respiratory Syncytial Virus (RSV)

(RSV) belongs to the family Paramyxoviridae. It infects the lungs' epithelium and the respiratory tract which causes serious respiratory diseases in children. There is no vaccine available for RSV. Its genome is a single RNA molecule of negative-sense RNA, which codes two surface glycoproteins (protein G and protein F) exposed on the viral envelope. G protein is responsible for receptor binding protein, and F protein serves for the fusion between the cell membrane and the viral envelope. F protein is expressed on the cell surface and fuses adjacent cells and makes syncytia formation, a well known cytopathic effect (52). Silver nanoparticles are being utilized to study the inhibition of RSV. The capping agents are 1) poly(N-vinyl-2-pyrrolidone) (PVP); 2) bovine serum albumin (BSA); and 3) a recombinant F protein from RSV (RF 412) for the silver nanoparticles (50). The TEM analysis indicates that the 1) BSA-conjugated silver nanoparticles interact with RSV without exact organization or spatial arrangement; 2) RF 412 coated silver nanoparticles are suspended liberally without regular attachment; and 3) PVP-coated silver nanoparticles bind to the viral surface that has regular spatial arrangement and attached G proteins. They interact with the G proteins and get dispersed on the RSV virions. When cells interact with BSA, PVP and RF-412 coated silver nanoparticles, inhibition is categorized by immunofluorescence microscopy, no inhibition is shown by BSA and RF-412 coated nanoparticles, whereas PVP coated silver nanoparticles inhibit 44% infection (50).

Sexually Transmitted Diseases (HSV-2, HIV)

The cellular uptake of HSV-2 and other herpes viruses through clathrin-mediated endocytosis can be achieved by interactions with cell receptors. The similar mechanism of intracellular internalization further shows the possibility of intracellular interactions (56). Results indicated that although a 100 μg/mL or higher Ag-NP concentration was toxic to Vero cells, 50 and 25 mg/mL Ag-NPs could significantly inhibit the generation of HSV-2 progeny.

HSV-2 infection can easily cause ulceration of the genital tract, thus facilitating the spread of HIV and other sexually transmitted diseases. One of the drugs used to treat vaginal infections is nonoxynol-9, which can inactivate viruses through the destruction of the viral envelope protein. However, nonoxynol-9 is a kind of surfactant that damages vaginal epithelial cells, causing vaginal dysbacteriosis, and facilitating the spread of diseases such as HIV. Short interfering RNA inhibitors and other mucosal microbial drugs have not yet been launched on the market because they degrade easily and have toxic effects (Katakowski and Palliser, 2010). In China, India, and other countries, silver and silver derivatives are often used in traditional medicine as antibacterial and antiviral preparations, for example the widely used silver sulfadiazine is a nanosilver preparation. In the present study, they used the MTT method to show that AgNPs at concentrations less than 100 μg/mL have very limited toxicity to cells while inhibiting the replication of HSV-2. Therefore, nanosilver is a promising drug for use against sexually transmitted diseases (56).

A previous study demonstrated that AgNPs preferentially combined with the glycoprotein gp120 of HIV (Elechiguerra et al., 2005). In the present study, the AgNPs may have formed bonds with a glycoprotein membrane of HSV-2 that contains a sulfhydryl group, which can strongly interact with AgNPs. This interaction may prevent internalization of the virus by inhibiting the interaction between the glycoprotein and a receptor. Cellular uptake of Ag- NPs occurs mainly through clathrin-mediated endocytosis and macropinocytosis (Asharani et al., 2009).

Enterovirus

EV-68 is a member of the Picornaviridae family, an enterovirus. First isolated in California in 1962 and considered rare, it has been on a worldwide upswing in the 21st century (58, 59, 60).

EV68 is one of the more than one hundred types of enteroviruses, a group of ssRNA viruses containing the polioviruses, coxsackieviruses, and echoviruses. Children less than 5 years old and children with asthma appear to be most at risk for the illness (62) although illness in adults with asthma and immunosuppression have also been reported (61). A 21 month-old toddler in Michigan died from the virus on October 10, 2014. The virus has sickened 691 people in 46 states and Washington, D.C., according to the CDC. Several people have also exhibited neurological symptoms similar to polio, although the enterovirus is considered a non-polio strain.

There is no specific treatment and no vaccine, so the illness has to run its course; treatment is directed against symptoms. Most people recover completely, and few need to be hospitalized (61).

The US Centers for Disease Control and Prevention (CDC) recommend "avoiding those who are sick." Since the virus is spread through saliva and phlegm as well as stool, washing hands is most important (63).

Chikungunya Virus (mosquito transmission)

Chikungunya virus was one of the first viruses researched as a possible biological weapon because of its ease of transmission (57).

Most people infected with chikungunya virus will develop some symptoms usually begining 3–7 days after being bitten by an infected mosquito. The most common symptoms are fever and joint pain. Other symptoms may include headache, muscle pain, joint swelling, or rash. Chikungunya disease does not often result in death, but the symptoms can be severe and disabling. Most patients feel better within a week. In some people, the joint pain may persist for months or years. People at risk for more severe disease include newborns infected around the time of birth, older adults (≥65 years), and people with medical conditions such as high blood pressure, diabetes, or heart disease.

Once a person has been infected, he or she is likely to be protected from future infections. There is no cure to treat chikungunya virus infection or disease, but symptoms are treated by getting plenty of rest, drinking fluids to prevent dehydration, taking silver nanoparticles three times a day and medications to relieve fever, pain and inflammation (CDC 2014).

Summary and Conclusions

Viruses are the number one cause of disease, disability and death worldwide. Viruses are incomplete segments of genetic material contained within a viral capsid that relentlessly seeks a healthy cell to attach to, fuse with, infect and multiply. Because viruses are neither alive nor dead, they cannot be killed. They transfer genetic material in a hurtful and fatal way.

The most notable finding from this paper is that there is no cure for viral disease. The CDC recommends vaccination, prevention through hygiene, pain relief and if you survive a

viral disease you have immunity. Vaccines have serious side effects and potential effects that are worse than the disease at times. Antiviral drugs have serious potential side effects and cannot be used in everyone. It is obvious that the immune system can prevent, cure and eliminate viral infections; however, we are placed under tremendous stresses from the microenvironments we live in. This stress compromises our immune system's ability to keep up with the demands placed upon us as evidenced by the increasing number of autoimmune and chronic diseases.

It is my conclusion and recommendation that in addition to preventive hygiene, and medication that the use of silver nano particles should be used everyday. Silver nanoparticles have been demonstrated to block viral entry, as well as all other viral effects and protect our healthy cells from viral disease. Regardless of the virus genus or species if viral cells cannot bind to the surface of healthy cells the virus is rendered ineffective. In addition, silver is beneficial to the immune system and because silver can destroy bacteria, viruses and yeast, it is the one unique product capable of preventing and treating secondary infections that afflict immune compromised, virally infected patients.

In most viral infections, the first symptom is immune deficiency and fatigue. This leaves many patients susceptible to streptococcus, staph and other opportunistic diseases that can be more fatal than the virus. For this reason silver nano particles can be used to prevent the secondary infection and the virus simultaneously.

Influenza is prevented using silver in a preventive treatment, and any other virus could be prevented by silver if the attachment step is blocked by silver. Silver has been shown to synergize with antibiotics producing as much as a ten fold increase in antimicrobial activity. Until we find the perfect medical treatment silver nanoparticles combined with proper hygiene, and preventive or treatment vaccines and drugs, makes our arsenal against viruses much stronger than ever before. The science on viruses using silver nano particles clearly demonstrates the destruction of bacteria, yeast and viruses, and can be used preventively or for treatment. The safe and broad spectrum benefits of silver combined with the published mechanism of action that prevent viral attachment gives hope where there are no treatments for current or mutating viruses.

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