WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 12, 2240-2250. Review Article ISSN 2277– 7105

FUTURE PROSPECTS OF COLLOIDAL NANOPARTICLES FOR MANAGEMENT OF INTRACTABLE BLOOD-STREAM INFECTIONS: A REVIEW

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Article Received on
20 July 2022,

Revised on 10 August 2022,
Accepted on 30 August 2022

DOI: 10.20959/wjpr202212-25474

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ABSTRACT

Intractable blood-stream infections by multi-drug resistant microbes and cancers are two major challenges for effective management by traditional medicines. Currently available all heavy-metal based colloidal nano-particles (NPs) elicit strong evidence of anti-bacterial, anti-fungal, anti-viral and anti-cancer effects by in-vitro studies for unique multi-targeted mechanism of action; but are failing to be approved drug for unacceptable cytotoxicity, geno-toxicity or immuno-toxicity to the host or environmental microbes. Toxicity can be selectively reduced, exploiting their property of instant attachment to target cells with surface charge differences and selective internalization. NPs initiate PCD after interaction with reduced heavy metal atoms through excess ROS production, only after access to the cells. An effective novel class of NPs, free of such side effects has been designed, after understanding PK-PD of such products. This has been possible by using compatible serum as capping material for reduced atomic aggregations of silver (Ag^0). Advantages of such NPs are prompt binding with the targets without quick degradation of bio-efficacy due to absorption of further stabilizing different plasma components as "corona protein caps". After small rational use of self-plasma protein capped NPs, almost total consumption is possible by rapidly multiplying target cells with high negative surface charge. There remains no scope for inducing toxicity to host cells with lower surface charge differences or receptor-ligand inadequacy for endocytosis. Initial studies indicate their potent target specific action and wide margin of safety by in-vitro and in-vivo studies. Synergistic use of traditional drug may further reduce safe required dose.

KEYWORDS: Silver Nanoparticles, Anti-microbial activity, Margin of safety, Therapeutic prospect.

The target specificity of heavy-metal based, pan-cytocidal colloidal nanoparticles have been made selectively toxic to rapidly multiplying microbes by redesigning novel class nanoparticles, using host's serum as capping agent on surface. For their unique mechanism of action, they may appear as potent, resistance-proof, nonspecific antimicrobial agents.

INTRODUCTION

Need for suitable alternative antimicrobials[1] in the context of evading drug resistance mechanisms by microbes, have become imperative. Most of conventional antimicrobial agents are effective on a few specific target molecules of replicating microbes, leaving opportunity for evolving resistant strains by genetic adaptation under selective pressure. By this way drug-resistance problem has become a great threat to infection control strategies. In search of resistance-proof, potent alternative agents, heavy-metal based colloidal nanoparticles (NP) now are in the front-line[2]; in particular the colloidal silver nanoparticles (AgNPs). Their unique mechanisms of multimode nonspecific actions,[3] irrespective of resistant genes in microbes, have made them ideal candidate drugs by in-vitro studies. But these are failing to be approved drugs due to risk of unacceptable toxic effects on host cells or environmental microbes. Recently, after understanding reasons and mechanisms of such toxicity, appropriately designed NPs have been developed and tested for a safe systemic usable antimicrobial agent in future.[4] In view of changing bio-functional efficacy at some circumstantial conditions, re-evaluation is needed for translational research of most earlier version toxic NPs with the safe version.

At unusual materialistic state, the energy level of colloidal NP exhibits higher affinity towards oppositely charged cells and imparts potent cytotoxic property.[5] Ionised silver-nitrate ($AgNO_3$) solution form unstable aggregates of atomic silver (Ag0) in presence of suitable reducing substance and proper physical conditions. When these are stabilized by surfactant capping molecules, generate relatively stable nano-sized (average 1-100 nm) colloidal silver nanoparticles. The change of surface plasmon resonance to incident light is indicative of proper materialistic alteration. At this state NPs strongly attracted towards oppositely charged cells and invade cell membrane. After access into cell, NPs induce oxidative stress mediated apoptosis.[6] Through the nano induced damaged cell-membrane,

conventional antimicrobial agents also get easy access into cell and exhibit non-specific synergism.[7]

In-vitro and in-vivo susceptibility studies for carboxy-methyl cellulose capped 9.5 nm size AgNPs against all tested bacteria and yeast has shown> 100 folds potent anti-microbial actions of such unique materialistic product, than that of equivalent ionic silver.[8] Such enhanced antimicrobial characteristics has been named as "Bonus Effect" and treated as bio-parameter of the colloidal NPs. The approach has simplified quality assessment of anti-microbial NPs without need for costly and cumbersome physical characterization every time, using electron microscope, UV spectrophotometer, Zeta potentiometer etc. Their half-life period in terms of antimicrobial efficacy has also been determined by reduction of "Bonus Effect" with time of preservation. The product filled in an air-tight amber colour bottle, can be preserved up to 3-6 months in refrigerator without functional alteration. The non-specific synergism of the product with full range anti-bacterial and anti-fungal agents incorporated in automated susceptibility device (VITEK-2) has also been indicated by comparing a pair of tests for different drug resistant microbes, one as control, another with 1/4th MIC effective concentration AgNPs in emulsion fluid. However, this product is not for systemic use, though can be tried for topical application on eye or other wound infections.

The quantum of cytotoxicity by NPs depends upon size, shape and zeta-potential of the particle and accessibility of it into any target cell. The smaller is the size of a particular nano-product the greater is surface-volume ratio with higher binding probability to target cells. Due to high positive surface charge, AgNPs are attracted instantly towards rapidly multiplying cells with negative surface charge, resulting disintegration of membrane phospholipids arrangement. Following entry through those damaged membranes or following receptor-ligand mediated endocytosis, AgNPs are de-capped inside cell with release of Ag0. Then gradually, Ag0 interacts with different oxidant bio-molecules producing excess of reactive oxygen species (ROS) and ionic silver, which results programmed cell death (PCD). Same thing can happen with oxygen loaded RBC or rapidly multiplying normal host cells or cancer cells. Excess ROS may be released into circulation from damaged AgNP-loaded RBCs and endothelial cells. This can cause cytotoxicity, immunotoxicity and genotoxicity at various levels. Similarly, microbes present in soil and water-bodies may be at risk from excess spillage of such NPs. So, most AgNP preparations are non-approved drugs. Their potent antimicrobial activities primarily depend upon size, shape and zeta-potentials, while in-vivo

toxicities[9] to host cells depend upon de-capping and cascades of reactions following prompt affinity-based binding into some rapidly multiplying miss targeted host cells like, haemopoietic cells, immune-proliferative cells, reproductive and regenerating cells. It is also proved that unusual materialistic state of silver atoms in NPs is responsible for potent cytotoxicity, which is not inducible by use of equivalent ionic silver.[10]

Recently the gradual increase of size and decrease of surface charge have been demonstrated following exposure of such colloidal NPs to fluid components of blood due to further adsorption of many high affinity proteins as secondary corona protein cap.[11] So, their bio-efficacy is likely to alter according to newer physical parameters and surface affinity molecules. Hence bio-efficacy of all earlier tested colloidal NPs need to be re-evaluated after in-vitro exposure to plasma proteins which can represent true PK-PD of such NPs in our body. Some workers[12] have evaluated in-vitro blood compatibility of PVP and Citrate capped AgNPs and showed dose dependent haemolysis, lymphocyte proliferation, coagulation with platelet aggregation and complement aggregation. They have also demonstrated adsorption of different plasma proteins as corona-cap on AgNPs. Results suggest instant affinity-based targeting of blood cells before acquiring secondary corona protein cap and a reason for toxicity to the host. Exploiting this phenomenon, suitable smaller size, stable, safe systemic-usable nanoparticles have evolved[4] by use of host serum as primary capping agent, intending to avoid development of additional protein corona cap in circulation, as well as to confer a host compatible product without recognisable foreign protein structures. Toxicity assessments with such pooled human serum capped AgNPs 17 nm size along with 13 nm size poly-vinyl alcohol capped AgNPs have shown maximum tolerable concentration of former in laboratory mice, which are found to be much higher than MIC values of all tested multi-drug resistant bacteria and yeast isolates; while same is not true for latter. Toxicity assessment on liver cell-line also reiterates the fact. For their potent, resistance-proof mechanisms of action irrespective of cell replication cycle, these may be sufficient to manage intractable systemic infections at small non-repetitive dosage with or without synergistic application of conventional antimicrobials. In a projected calculation it has been shown that, the achievable plasma concentration of such novel NPs in a 100 kg adult individual after infusion of 100 ml nano product, will be about 5 MIC of most MDR strain bacteria. Required dose of nano-product may be as low as 10 ml, by synergistic use of an appropriate apparently resistant antimicrobial drug. To prepare that amount of AgNPs, only 2.5 ml of patient's serum is required.

Most AgNPs are also anti-biofilm[13] in nature for their greater penetration into biofilm matrix and targeting dormant non-replicating cells within. For common mechanisms of action, it is expected that safer version NPs with comparable size, shape, zeta-potentials, will also be equally effective like earlier version NPs against infections by viruses[14,15] or parasites.[16] Repeat testing with safer version novel NPs is required in this aspect. After several phase trials, such novel AgNPs can be used as pan-microbicidal drug with a rational therapeutic dose and may appear as "super magic-bullet" in post-penicillin-era"! Multi-mode mechanisms of action unrelated to genetic control, will not only make NPs resistance-proof but also may help to combat increasing anti-microbial resistance problems by synergistic use.

Novelty of such nanomedicine is that, it may obviate need for specialist's opinion to start early empirical use without aetiological diagnosis of infective agents by costly investigations. Same can be a ready solution for combating new emerging infections in man or animals. Added benefit may be in the management of infections in cancer patients, because same drug also possesses nonspecific anticancer effects for targeting rapid-multiplying cells with slightly different surface molecular identity. A group of workers[17] have tested efficacy of AgNPs capped with mouse serum albumin in mice fibrosarcoma model and demonstrated additional mechanism of immune-modulation towards anti-tumour effects. Earlier attempts for targeted drug-delivery using carbon-based nano-structures tagged with single selected antimicrobial, can't be substitute of omnipotent, resistance-proof product like colloidal NPs. Heavy metal based colloidal NP itself is oxidative stress producing non-specific cytocidal, while efficacy of single drug carrying carbon-based nano-conjugate is limited to the susceptibility of the original drug. Scientists[18] have tested antifungal activity of functionalized carbon nano tubes (f-CNT) conjugated with Amphotericin B (AMB), along with AMB and AMB-Deoxycholate. They have concluded either comparable or slight better antifungal activity of f-CNT carried AMB. Probably such nano based targeted drug delivery will not be cost-effective, and safe for environmental microbes due to unpredictable nonspecific affinity to different bio-molecules by the excreted CNT.

The long-term potential risks to the host or to the environmental microbiota should also be evaluated before therapeutic use of NPs. In last 2-3 decades, thousands of different NPs have been prepared and tested in-vitro, without considering important pharmaco-kinetic aspect of changing materialistic behaviours after exposure into body fluid or degrading agents. Some of these are indicated for topical applications or indwelling medical device coating. However,

questions have risen about adverse systemic side effects by absorbed number of NPs. Several workers have prepared NPs by green synthesis,[19] using various plant or microbial extracts as capping materials. These are mainly intended for their use as disinfectants, preservatives or pesticides without considering impact on environmental microbes after spillage of excess.[20] For assessment on adverse environmental impact from excess use of NPs, a study has been conducted and concluded dose dependent immune-toxicity by citrate capped 20 nm AgNPs on leukocytes of marine mammal, Dolphin at high concentrations of 10 and 50 µg/ml.[21]

Some workers have reported high safety level of bovine serum albumin (BSA) capped AgNPs[22] tested against murine salmonellosis model. Others[23] have reported similar experience using gel of BSA capped AgNPs against *Staphylococcus aureus*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*. Scientists have reported 30 times higher margin of safety in albumin capped AgNPs tested on human breast cancer cell lines.[24] Higher margin of safety may be partially true, because still chances remain for attaching many other small plasma proteins on circulating NPs, which may result bio-functional degradation and some risk for targeting host cells before such degradation. Ideal system friendly NPs can be prepared, only using self-plasma as capping agent which contain iso-antibody matched cocktail of capping agents. However autoimmune diseases can be precipitated. In that case, auto-antibody-free homologous plasma from donors remains as an alternative. These can be in-house prepared following some simple steps of mixing in appropriate volume and physical conditions.[4] Automated vending machine can also be developed for mass scale use. Addition of infection specific anti-sera can improve specificity of the product. Though these are indicated for intractable blood-stream infections, but limitations remain for intra-cellular parasites or spores.

Following oral use of AgNPs chance of their quick degradation into ionic silver with exposure of gastric acid or de-capping enzymes, expected antimicrobial action may not be evident. Testing on rat after feeding oral dose of PVP capped AgNPs and equivalent silver acetate for 21 days, a group of scientists have noted similar distribution of silver in gut, liver and kidney.[25] Some other workers have carried time exposure study in 60 human volunteers feeding with 10 to32 ppm oral dose of AgNPs and observed no significant physiological alteration.[26] However weak antimicrobial action has been observed due to released heavy metals with varying degree of toxicity and dysbiosis. Similarly, use of NPs by intra-muscular route may have limited value for delayed contact with target microbes, before degradation in

presence of myo-globulins or other oxidative radicals. Direct injection into lesion maybe rewarded, particularly in case of growing tumour.

When AgNPs are prepared using single type of capping material, instead of a cocktail of attachable proteins, the size selection, agglomeration and antimicrobial action of AgNPs are controlled by optimum concentration of capping agent.[27] It is also noted that the agglomeration of NPs caused by the polymer bridging mechanism is stronger by anionic dispersant than cationic dispersant capping agents. Un-capped nano aggregates of heavy metal atoms tend to fix with many larger organic molecules in appropriate physical conditions to attain maximum stability. Otherwise, these tend to agglomerate into larger particles with exposure to light, oxygen or any oxidant molecules. So also, gradual transformation continues after topical application, yielding marginal benefit caused by instant attachment of some NPs with microbes. While, such applications can create adverse effects to the regenerating cells of the wound or immune cells, even at sub-toxic dose. Some workers recommended topical use of NPs in gel form[28], so that slow sustainable release of drug is ensured with lower chance of exposure to light or oxygen. However, novel serum capped AgNPs if not used in excess, can be quickly taken up by microbes in circulation and small residue may be gradually transformed into ionic silver before elimination from system. Several phases of trials and PK-PD assessments are required before their use as drug.

The prospect of self-contained anti-microbial and anti-cancer properties of colloidal NPs is based on sufficient theory testing and laboratory experiments in support of medical applications. Studies by a group of scientists[29] have shown that charge signs of tested all 22 cancer cells of different organs are strongly negative with high affinity for positively charged nanoprobes, in contrast to corresponding normal cells with positive or weak negative charge signs. The high rate of glycolysis has been shown proportionate to negative surface charges of cancer cells. Glucose metabolism dependent excess ROS production by AgNPs inside Hepatoma cells, is also reported.[30] Same metabolic character of rapidly multiplying microbes may be related with their high negative charges and affinity for AgNPs. High affinity and permeation of SiO_2 nanoparticles within the biofilm matrix has also been observed.[31] However progressive decline of negative surface charge density of herpes simplex infected cells is reported with increased production of progeny viruses.[32] Recently, in-vitro entry level inhibition of SARS-CoV-2 infection by different sizes and concentrations of AgNPs has been reported with evidence for disruption of viral integrity.[14] So, safe version

of AgNPs has limitation for arresting most of intra-cellular viruses. Further tests are to be done to note any evidence for entry of NPs by indirect mode like endocytosis of nanoparticles tagged with viral envelop or binding with viral antigen expression sites of infected cells.

The efficacy of safe NPs can be increased after tagging with infection specific antibodies.[33] Such adsorbed antibodies on capping agents of AgNPs can be used for high affinity specific attachment to target cells. According to scopes and limitations of nano-medicines for management of intractable blood-stream infections, antimicrobial tagged carbon based nano structures can be considered as 1st generation nano-medicne, colloidal heavy metal based nanoparticles as 2nd generation, while compatible serum capped colloidal NPs as 3rd generation and same enriched with infection specific antibody as 4th generation drugs. However, nano-medicines should be very restrictively used at a rational minimal dose, preferably with synergistic drugs. For cancer management a sub-lethal sensitizing dose may be tested for synergistic use with chemotherapy or radiotherapy to reduce toxicity and cost. Trials may be conducted on management of animal cancers using self-plasma capped AgNPs. Presently available nano-products for biomedical applications[34] should be re-evaluated and replaced by safer version nano products. As colloidal AgNPs have tendency to react slowly with anti-oxidants, drug-drug interactions should be studied before their use.

ACKNOWLEDGEMENT: Nil.

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