http://jbcp.shahed.ac.ir 10.22070/jbcp.2019.3672.1105

Open Access Research Article

Journal of Basic and Clinical Pathophysiology
Print ISSN: 2322-1895, **Online ISSN:** 2345-4334
Vol.7, No.1, March 2019

A Semiannual Publication of
Shahed University Press

Antimicrobial effect of zinc oxide and silver nitrate nanoparticles against *S. aureus*, *A. baumannii* and *P. aeruginosa*

Mohammad Niakan[1], Iman Pouladi[1*], Rasoul Kaviani[1], Elham Esmaili[2]

1. Department of Medical Microbiology, Faculty of Medicine, Shahed University of Medical Sciences, Tehran, Iran.
2. School of Medicine, Shahed University, Tehran, Iran

Abstract

Background and Objective: Nanoparticles have been introduced as novel antimicrobial agents because of their properties that are different from their bulk properties. Present study was aimed to investigate antimicrobial activity of silver nitrate and zinc oxide nanoparticles against three main bacteria responsible for nosocomial infections, S. aureus, P. aeruginosa and A. baumannii.

Materials and Methods: Solutions of nanoparticles were prepared at various concentrations (31.5-4000 ppm) in a serial method. Disks with various concentrations of nanoparticles were then placed on bacterial cultures for 24 hours and diameter of inhibition was measured after 24 hours of exposure to nanoparticle in incubator. Using a diagram without statistical analysis, diameters of inhibition were compared between various concentrations and kinds of bacteria. Analysis of variance was used to compare the diameter of inhibition between bacteria based on a variety of nanoparticles regarding their concentration.

Results: Nanoparticles of zinc oxide made an inhibitory diameter of 13.6 mm at highest concentration to 7 mm at lowest concentration of nanoparticle for S. aureus. For this bacterium, silver nitrate nanoparticle had a larger inhibitory diameter (16.33 mm to 8.67 mm). Zinc oxide nanoparticle did not have an inhibitory effect on P. aeruginosa and A. baumannii. The maximum inhibitory diameter of silver nitrate nanoparticle on P. aeruginosa and A. baumannii was measured 13.33 mm and 22.67 mm for P. aeruginosa and A. baumannii, respectively. For both bacteria, inhibitory area reached to zero at a concentration of 125 ppm. Inhibitory areas of silver nitrate were significantly greater than those for zinc oxide ($p<0.001$).

Conclusion: In summary, silver nitrate nanoparticles have greater antimicrobial activity. Antimicrobial activity of zinc oxide nanoparticles was restricted to gram-positive bacteria.

Key words: Antimicrobial activity, Nanoparticles, Silver nitrate, Zinc oxide, S. aureus, P. aeruginosa, A. baumannii

1. Introduction

Nosocomial inactions are defined as infections without evidence of incubation at the time of admission to a healthcare setting. It can be said that two main challenges of the infectious disease medicine are facing resistance to antibiotics and nosocomial infections in the current century (1). Widespread usage of antibiotics and high potential to overcome biological targeted agents encourage scientists to develop newer versions of antimicrobial agents such as nanoparticles. Nanoparticles have been introduced as novel antimicrobial agents because of their properties that are different from their bulk properties (2). Antibiotic resistance is a problem of global significance. Moreover, the overuse of antibiotics causes the emergence of bacterial resistance and increases healthcare costs and sepsis-related deaths. Widespread usage of antibiotics and high potential to overcome biological targeted agents encourage scientists to develop newer antimicrobial agents like nanoparticles (2-4). Nanotechnology has been taken global interest in nanoparticles (NP) that are of unique properties as compared to their bulk equivalents. The antimicrobial activity of NP was

Revised: November 2018, **Accepted:** February 2019, **Available online:** March 2019
Corresponding Author: Iman Pouladi **Email:** imanpouladi96a@gmail.com

previously illustrated and Ag and Zn are also described to destroy bacterial membranes in their nanoparticle forms. Then, nano-Ag destabilizes the outer membrane of E. coli, interfering membrane activities like ATP metabolism. Additionally, nano-Ag treatment makes pits in E. coli cell walls. Finally, nanoparticles of Ag interact with membrane integrity (1-4). Traditionally, antiseptic properties of silver had been known. Until the development and widespread usage of antibiotics, dilute solution of silver nitrate was used for infants' eyes at birth to prevent gonorrhea infection transmitted from mothers' genital tract. Eye infections and blindness of newborn were reduced by this method (5). Zinc oxide nanoparticles can improve the antibacterial activity of ciprofloxacin. It has been reported that nano ZnO with the average size between 20 nm and 45 nm can increase the antibacterial activity of ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli in vitro*. It has been indicated that enhancing effect of this nanomaterial is concentration-dependent against all tested strains. To explain that zinc oxide nanoparticles can interfere with NorA protein. NorA is a protein developed for conferring resistance in bacteria and has pumping activity that mediate the efflux of hydrophilic fluoroquinolones from a cell. Another explanation is that zinc oxide nanoparticles can interfere with Omf protein. Omf is a membrane protein responsible for the permeation of quinolones into the cell (6). Thus, the present study was aimed to investigate antimicrobial activity of silver nitrate and zinc oxide nanoparticles against the three main bacteria responsible for nosocomial infections, *S. aureus*, *P. aeruginosa* and *A. baumannii*.

2. Materials and Methods

In this *in vitro* study, nanoparticles of silver nitrate and zinc oxide with an average size of 50 nm were procured**.** The structure of the particles was characterized by a powder X-ray diffraction (XRD) equipment (D/max2000, Rigaku) using Cu Ka radiation. Most agents used in this study were purchased from Sigma-Aldrich. The fluorescent reporter dye 39-(p-hydroxyphenyl) fluorescein (HPF) was purchased from Invitrogen, *Staphylococcus aureus* (ATCC:25923), E. coli O157 (ATCC:43895), *S. aureus* (ATCC:25923), *P. aeruginosa* (ATCC:27853) and *A. baumannii* (ATCC:17978) were obtained from the American Type Culture Collection from the Korean Collection for Type Cultures. Solutions were prepared in various concentrations (4000 ppm to 31.5 ppm) in a serial method.

2.1. Microbiological assay

The experimental procedure was performed according to standard methods. Culture medium, agar plates, PBS, and distilled water were prepared and sterilized before running antibacterial sensitivity assay. To prepare Mueller-Hinton (MH) broth (Difco), 10 g/l of sodium chloride, 5 g/l of yeast extract and 10 g/l of tryptone/peptone from casein were weighted and placed into a precipitation glass. Then, distilled water was poured and the system was magnetically stirred up until dissolution. The broth was then autoclaved at 121ºC for 20 min and subsequently stored at 2-8ºC for no more than 15 days. To prepare MH agar plates, 14 g/l of agar-agar was weighed and added to other ingredients to solidify the MH medium. The liquid was quickly poured onto petri dishes. Agar solidifies below 60ºC; petri dishes were cooled down at room temperature and then stored at 2-8ºC. Phosphate buffer saline (PBS) was prepared by mixing 8 g/l of sodium chloride, 0.2 g/l of potassium di-hydrogen phosphate anhydrous, 0.2 g/ l of potassium chloride and 1.15 g/l of di-sodium hydrogen phosphate anhydrous. All these salts were weighed and dissolved in distilled water. Using turbidity of 0.5 McFarland, standard *S. aureus* (ATCC:25923), *P. aeruginosa* (ATCC:27853) and *A. baumannii* (ATCC:17978) were cultured for 18 hours at 37°C in Muller Hinton media. The antibacterial activity of nanoparticles was also evaluated under visible light illumination. Spectrophotometry were explored to assess bacterial growth because spectrophotometric readings are generally more precise and reproducible (7-9).

Blank disks were used to prepare 5 mm diameter disks which were used for testing each material. A measure of 1 mg of ZnO was spread on top of the disks followed by 10 µl of bacteria strain at a concentration of 109 CFU/ml. Disks with various concentrations of nanoparticles were then placed on bacterial cultures for 18 hours at 37°C. Each concentration of nanoparticles was also applied 3 times for each bacterium inoculates. Having been exposed to nanoparticle in incubator for 24 hours, the diameter of inhibition was measured. Means of 3 cultures with same concentration and a kind of nanoparticles and bacteria were considered for analysis. Using a diagram without statistical analysis, diameters of inhibition were compared between various concentrations and kinds of bacteria. Analysis of variance was used in order to compare the diameter of inhibition between bacteria based on kinds of nanoparticles regardless of their concentration. Results were analyzed statistically using one-way ANOVA followed by Tukey test.

3. Results

This study was done to evaluate the antimicrobial effects of zinc oxide and silver nitrate nanoparticle solution against three strains of *S. aureus*, *P. aeruginosa and A. baumannii* bacteria. The results of growth inhibition effect of used nanoparticle solutions against three standard bacteria are presented in Figures 1 and 2 and Tables 1 and 2. Nanoparticles of zinc

oxide made an inhibitory diameter of 13.6 mm in the highest concentration to 7 mm in the lowest concentration of nanoparticle in *S. aureus* (Fig. 1, Table 1). For this bacterium, silver nitrate nanoparticle had a larger inhibitory diameter (16.33 mm to 8.67 mm) (Fig. 2, Table 1). Zinc oxide nanoparticle had no inhibitory effect on *P. aeruginosa* and *A. baumannii*. The maximum inhibitory diameter of silver nitrate nanoparticle on *P. aeruginosa* and *A. baumannii* were measured 13.33mm and 22.67 mm for *P. aeruginosa* and *A. baumannii*, *respectively*. For both bacteria, inhibitory area reached to zero at a concentration of 125 ppm (Tables 1 and 2). Inhibitory areas of silver nitrate were significantly greater than zinc oxide ones ($p<0.001$).

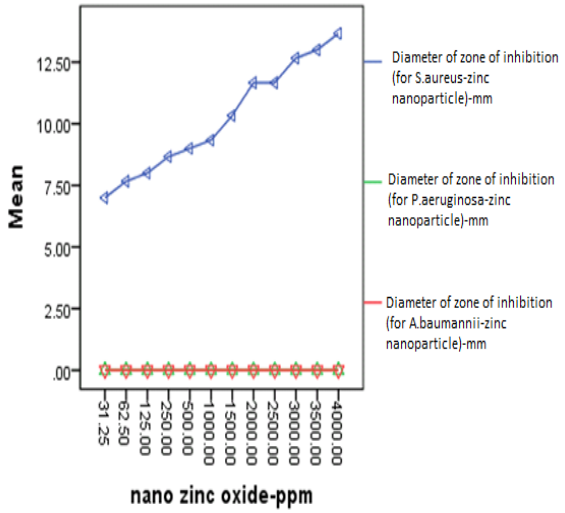

Figure 1. Zone of inhibition in various zinc oxide nanoparticle concentrations. *P. aeruginosa* and *A. baumannii* growth was not inhibited by zinc oxide nanoparticles.

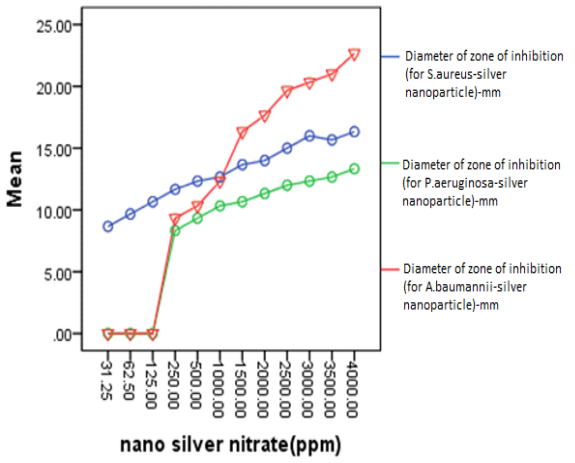

Figure 2. Zone of inhibition in various silver nitrate nanoparticle concentrations.

Table 1. The results of bacterial growth inhibition of zinc oxide and silver nitrate nanoparticles

Diameter of zone of inhibition (mm)		Minimum	Maximum
Zinc oxide nanoparticles	*S. aureus*	7.00	13.67
	P. aeruginosa	0.00	0.00
	A. baumannii	0.00	0.00
Silver nitrate nanoparticles	*S. aureus*	8.67	16.33
	P. aeruginosa	0.00	13.33
	A. baumannii	0.00	22.67

Table 2. Comparison of zinc oxide and silver nitrate nanoparticles in bacterial growth inhibition

Difference between zinc oxide and silver nitrate nanoparticles zone of inhibitions	Minimum	Maximum	Mean	Std. Deviation	P value
S. aureus	1.67	3.3	2.80	0.5	0.00
P. aeruginosa	0.00	13.3	8.36	5.2	0.00
A. baumannii	0.00	22.6	12.47	8.5	0.00

4. Discussion

According to less done clinical study, inhibiting bacterial growth by applying nanoparticles is not a novel idea, though using nanoparticles are not popular in patients' treatment up to now. Novelty of our study is the consideration of bacteria responsible for nosocomial infection. *S. aureus*, *P. aeruginosa* and *A. baumannii* are the most prevalent bacteria isolated from nosocomial infections, predominantly respiratory and skin infections (10). Although most laboratory studies have demonstrated antibiotic properties of many nanoparticles, but the different behavior of bacteria in the body and the complex interactions between body and pathogen, prevent the use of them prior to further studies. The mechanism of nano-medicines action with antibiotic properties is not dependent on biological processes that target older antibiotics, so probably we will face less resistance (11). The results showed that silver nitrate nanoparticles hade more antibacterial activity on the studied bacteria. It has been previously indicated that silver nitrate has antibacterial activity against a variety of bacteria (10). Releasing free electrons has been recommended for antimicrobial activity of silver nitrate nanoparticles as a useful mechanism (12-15). Among published studies on antimicrobial activity of silver nitrate, absence of evidences about *in vivo* usage has been observed in animal laboratories. Silver is a toxic element and accordingly it is not acceptable to be used *in vivo* (16). Studying antimicrobial activity of zinc oxide nanoparticles has been less focused. So, further studies may demonstrate more about its antimicrobial activity. In our study, zinc oxide nanoparticles have no antimicrobial activity against gram-negative bacteria (7,8,17,18). Nanoparticles

have potentials to be used *in vivo*, but less is known about probable toxic effects and more questionable ones, their effects on normal host cell cycle and metabolism. They may have a potential carcinogen effects. Up to know, studies are not in agreement about adverse effects of nanoparticles. They may be accumulated in body tissues leading to organ failure (19). In other studies, Hoseinzadeh and colleagues in 2012, examined the effect of zinc oxide (ZnO) nanoparticles on death kinetic of gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and gram-positive bacteria (*Staphylococcus aureus and Staphylococcus epidermidis*). In their study, ZnO nanoparticles had growth inhibition effect on all studied bacteria including pseudomonas bacteria and even more effective on gram-negative bacteria (9). However, in our study, ZnO nanoparticles did not cause any inhibition of growth for *Pseudomonas*. This difference in results may be due to differences in size of used ZnO nanoparticles in these studies. But from the view that by increasing the concentration of nanoparticles, the growth rate decreases, these two are the same direction (9). Dose-dependent responses of bacterial growth to nanoparticles recommend a pharmacologic mechanism for them. Releasing free radicals, directing destruction of the cell wall and membrane, inducing conformational alterations in cell wall proteins, impairing electron transferring chain and inhibition of metabolic pathways by changing enzymatic activities are potentially regarded as targets of nanoparticles. Like other classic antimicrobial agents, some of these targets are not distinguishable from host cell ones. Our study had some limitations. The main limitation was lacking a control of bacterial culture not to be exposed to nanoparticles.

In conclusion, silver nitrate nanoparticles have more antimicrobial activities. Antimicrobial activity of zinc oxide nanoparticles was restricted to gram-positive bacteria. The results of this study showed that a silver nitrate nanoparticle is effective on the growth of all three bacteria and can be used in further studies of nosocomial infections. Since the antibiotic resistance in *Staphylococcus aureus* and *Pseudomonas* are rapidly increasing, ZnO and silver nitrate nanoparticles can be considered as a treatment.

Acknowledgement

The ethics committee of Shahed University (Tehran, Iran) approved this study (IR.Shahed.Rec.1394/08).

References

1. Hajipour MJ, Fromm KM, Akbar Ashkarran A, Aberasturi D, Larramendi IR, Rojo T, et al. Antibacterial properties of nanoparticles. Trends in Biotechnology 2012; 30(10): 499-511.
2. Hernandez-Sierra JF, Ruiz F, Cruz Pena DC, Martinez-Gutierrez F, Martinez AE, Pozos Guillen A, et al. The antimicrobial sensitivity of *Streptococcus mutans* to nanoparticles of silver, zinc oxide, and gold. Nanomedicine: Nanotechnology Biology and Medicine 2008; 4(3): 237-240.
3. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for gram -negative bacteria. Journal of Colloid and Interface Science 2004; 275(1): 177-82.
4. Kim SH, Lee H, Ryu DS, Choi SJ, Lee DS. Antibacterial Activity of Silver-nanoparticles Against *Staphylococcus aureus* and *Escherichia coli*. Journal of Microbiology and Biotechnology 2011; 39(1): 77-85.
5. Dunn PM , Carl C. The prevention of ophthalmia neonatorum. Archives of Disease in Childhood-Fetal and Neonatal Edition 2000; 83: 158-159.
6. Banoee M, Seif S, Nazari ZE, Jafari P, Shahverdi HR, Moballegh A, et al. ZnO nanoparticles enhanced antibacterial activity of ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli*. Journal of Biomedical Materials Research 2010; 93: 557-561.
7. Medina C, Santos M, Radomski A, Corrigan O, Radomski M. Nanoparticles: Pharmacological and toxicological significance. Journal of Pharmacology 2007; 150: 552-558.
8. Samarghandi MR, Hoseinzadeh E, Alikhani MY. Susceptibility constant and death kinetic of *Escherichia coli* and *Staphylococcus aureus* to zinc oxide and copper oxide nanoparticles. Hamadan University of Medical Sciences and Health Services 2012.*(MSc thesis)* [Persian]
9. Hoseinzadeh E, Samarghandi MR, Alikhani MY, Asgari Gh, Roshanaei Gh. Effect of zinc oxide (ZnO) nanoparticles on death kinetic of gram negative and positive bacterium. Journal of Babol University of Medical Sciences 2012; 14(5): 13-19. [Persian]
10. Alizadeh M. Antibacterial effects of nanosilver on some hospital infections. Tabriz University of Medical Sciences 2011. (MSc thesis) [persian]
11. Lkhagvajav N, Yasa I, Celik E, Koizhaiganova M, Sari O. Antimicrobial activity of colloidal silver nanoparticles prepared by sol-gel method. Digest Journal of Nanomaterials and Biostructures 2011; 6: 149-154.
12. Aruna JK, Arunachalam J. Assessment of antibacterial activity of silver nanoparticles on *Pseudomonas aeruginosa* and its mechanism of action. World Journal of Microbiology and Biotechnology 2010; 1007(10): 569-677.
13. Amirulhusni A, Palanisamy NK, Mohd-Zain Z, Jian Ping L, Durairaj R. Antibacterial effect of silver nanoparticles on multi drug resistant *Pseudomonas aeruginosa*. World Academy of Science Engineering and Technology 2012; 67: 258-261.
14. Niakan S, Niakan M, Hesaraki S, Nejadmoghaddam MR, Moradi M, Hanafiabdar M, et al . Comparison of the Antibacterial Effects of Nanosilver With 18 Antibiotics on Multidrug Resistance Clinical Isolates of *Acinetobacter baumannii*. Jundishapur Journal of Microbiology 2013 ; 6(5): e8341.
15. Sintubin L, De Gussem B, Vandermeeren P, Pycke B, Verstracte W, Boon N. The antibacterial activity of biogenic silver and its mode of action. Journal of Microbiology and Biotechnology 2011; 31: 113-189.
16. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances 2009; 27(1): 76-83.
17. Raghupathi KR, Koodali RT, Manna AC. Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. Langmuir 2011; 27(7): 4020-4028.
17. Sotiriou GA, Paratsinis SE. Antibacterial activity of nanosilver ions and particles. Journal of Environment and Scientific Technology 2010; 15: 5649-54.
18. Choi O, Deng KK, Kim NJ, Rossjr L, Surampalli RY, Hu Z. The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. Water Research 2008; 42(12): 3066-3074.