

Antibacterial Effect of Silver Nanoparticles along with L-Arginine against *P. aeruginosa*

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Received : 11 Feb 2017

Revised: 09 May 2017

Accepted: 29 May 2017

ABSTRACT

Background and Objectives: Nowadays, the prevalence of multidrug-resistant pathogens such as *Pseudomonas aeruginosa* is increasing worldwide. Many studies have been seeking new treatment strategies to treat infections caused by these microorganisms. Silver nanoparticles (AgNPs) along with L-arginine have significant antimicrobial effects and could be used as alternatives for ineffective drugs.

Methods: In this study, the antibacterial activity of AgNPs, L-arginine and various concentrations of AgNPs along with L-arginine (12.5 and 25 mg/ml) were investigated against *P. aeruginosa* PA01 using the broth macrodilution method.

Results: Minimum inhibitory concentration of AgNPs, L-arginine and AgNPs combined with 25 and 12.5 mg/ml L-arginine was 15.6 µg/ml, 25 mg/ml, 1.9 µg/ml and 3.9 µg/ml, respectively. Minimum bactericidal concentration of AgNPs, L-arginine and AgNPs combined with 25 and 12.5 mg/ml L-arginine was 31.2 µg/ml, 50 mg/ml, 3.9 µg/ml and 7.8 µg/ml, respectively.

Conclusion: Our study suggests that AgNPs along with L-arginine can be used as an alternative antibacterial agent against *P. aeruginosa*, and might be useful for treatment of wound infections.

Keywords: Nanoparticles, Arginine, Anti-Bacterial Agents, *Pseudomonas aeruginosa*.

INTRODUCTION

Nowadays, the prevalence of multidrug-resistant pathogens such as *Pseudomonas aeruginosa* is on the rise (1). *P. aeruginosa* is involved in several infections such as urinary tract infection, pneumonia, meningitis and soft tissue infections (2, 3). Infections caused by resistant pathogens may increase the risk of treatment failure and severity of the disease (4). Resistance mechanisms in bacteria could be due to the indiscriminate use of drugs, ineffective infection control programs (5-7) and lack of novel therapeutic strategies against pathogens, especially *P. aeruginosa* (8, 9). Several studies have been conducted to develop new generation of drugs in order to overcome the threat posed by multidrug-resistant pathogens (10, 11). In this regard, silver nanoparticles (AgNPs) have been proposed as suitable alternatives that have good physicochemical and biological properties. They are simple to use, affordable and widely applicable in a variety of antibacterial methods (12). AgNPs also have acceptable antimicrobial activity (13-15) and high biocompatibility with far lower propensity to induce microbial resistance compared to current drugs. Due to these unique properties, these materials could be used as effective alternatives for elimination of multidrug resistant microorganisms (16-19).

L-arginine is an α -amino acid with antibacterial activity that has important roles in wound healing and urea cycle (20). The positive charge of L-arginine attract the negatively charged target membranes such as LPS in Gram-negative bacteria or teichoic acid in Gram-positive bacteria and phospholipid head groups (21). Because of the antibacterial effects of nanoparticles and L-arginine and also high tendency of arginine towards silver ions, it can be predicted that the silver-arginine complex show broader spectrum of antimicrobial effects (22, 23). Although AgNPs may have cytotoxic effects on host cell (24), these effects can be reduced by altering the therapeutic doses (25). Thus, reducing the concentration of nanoparticles via combination with L-arginine can be effective in minimizing the toxic effects of nanoparticles. Considering the limited number of studies on the interaction between arginine and metal ions (23), the present study evaluated the antibacterial activity of AgNPs along with

L-arginine against *P. aeruginosa* in an aqueous system.

MATERIAL AND METHODS

Subsequent dilutions (0.2 to 500 $\mu\text{g/ml}$) of colloidal AgNPs (average size 20 nm, Pishgaman Nano Arya Co.) were prepared in Muller Hinton broth from stock solution. Stock solution of L-arginine (Merck, Germany) was prepared in distilled water with final concentration of 200 mg/ml, and later used to prepare subsequent dilutions (0.1 to 100 mg/ml) in Muller Hinton broth, using serial two-fold dilution. Subsequent dilutions of AgNPs (0.2-500 $\mu\text{g/ml}$) were prepared in Muller Hinton broth. Two concentrations of L-arginine (12.5 and 25 mg/ml) were separately added to each tube.

P. aeruginosa PAO1 was obtained from Urmia University of Medical Sciences. The bacteria were grown in Muller Hinton broth at 37 °C for 24 h. Bacterial suspension equivalent to 0.5 McFarland standard (1.5×10^8 CFU/ml) was prepared in sterile normal saline (26).

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The MIC of AgNPs, L-arginine and AgNPs with L-arginine against *P. aeruginosa* was determined by broth macrodilution method according to the Clinical and Laboratory Standards Institute (CLSI, 2014).

Determination of MIC and MBC of AgNPs and L-arginine

Different concentrations of (0.2-500 $\mu\text{g/ml}$) of AgNPs were prepared in Muller Hinton broth using two fold serial dilutions. Bacterial suspension containing 1.5×10^8 CFU/ml was added to each tube, and the tubes were incubated at 37 °C for 24h. The MIC was defined as the lowest concentration of AgNPs that was able to inhibit bacterial growth. The dilution method was also used to determine MBC values. For this propose, 100 μl of bacterial suspension containing \geq MIC concentrations of AgNPs were cultured on Muller Hinton agar plates. After incubation at 37 °C for 24 h, the MBC value was defined as the lowest concentration of AgNPs that was able to kill 99.9% of bacteria. All experiments were performed in triplicate, on three different days (27). The MIC and MBC values for L-arginine were determined using the method

described for AgNPs by preparing different concentrations (100- 0.1 mg/ml) of L-arginine in Muller Hinton broth using two fold serial dilutions.

Determination of MIC and MBC of AgNPs along with L-arginine

First, bacterial suspension (1.5×10^8 CFU/ml) was added to tubes containing AgNPs along with L-arginine. The tubes were incubated at 37 °C for 24h. MIC and MBC of AgNPs along with L-arginine was determined using the method described for AgNPs and L-arginine. All experiments were performed in triplicate, on three different days.

RESULTS

The MIC of AgNPs, L-arginine and AgNPs along with two different concentrations (25 mg/ml and 12.5 mg/ml) of L-arginine was 15.6 µg/ml, 25 mg/ml, 1.9 µg/ml and 3.9 µg/ml, respectively. The MBC of AgNPs, L-arginine and AgNPs along with L-arginine (25 mg/ml and 12.5 mg/ml) were 31.2 µg/ml, 50 mg/ml, 3.9 µg/ml and 7.8 µg/ml, respectively (Table 1). Overall, the 20-nm AgNPs and L-arginine showed antibacterial activity against *P. aeruginosa* strain PAO1. The addition of 25 mg (MIC value) and 12.5 mg (MIC50 value) of L-arginine to various concentrations of AgNPs increased the antibacterial activity.

Table 1 -MIC and MBC values of AgNPs, L- arginine and AgNPs along with L- arginine against *P. aeruginosa*

Drug	MIC (µg/ml)	MBC (µg/ml)
AgNPs (µg/ml)	15.6	31.2
L-arginine (mg/ml)	25.0	50.0
NPs + 25 mg/ml L-arginine	1.9	3.9
NPs + 12.5 mg/ml L-arginine	3.9	7.8

DISCUSSION

Considering the growing prevalence of multidrug-resistant microorganisms and the subsequent increase in therapeutic expenses, several studies have sought safe treatment strategies to overcome antibiotic resistance (28). In recent years, nanoparticles have been extensively used in drug delivery (29) and therapies (30). Several recent studies have shown the antimicrobial applications of AgNPs (31-33). Although their mechanism of action is not yet clear, some studies have reported the positive charge of nanoparticles (34-36) and the excess production of free radicals on the surface of Ag (37) as the main inhibitory mechanisms. In the present study, we assessed the antibacterial effect of different concentrations of 20-nm AgNPs alone and along with L-arginine against *P. aeruginosa* PAO1 using broth macrodilution method. Our results showed that AgNPs had good antibacterial activity at concentration of 15.6 µg/ml. Similar to our study, many studies have demonstrated the antibacterial effect of AgNPs on Gram-positive and Gram-negative bacteria (38-40). Although AgNPs had good antibacterial effects, they may also cause significant cytotoxicity (33). In fact, finding a material with excellent antimicrobial properties and very low toxicity on the host cells is challenging (41). The cytotoxic effects

of AgNPs depend on some factors including dose, size and shape (42). Fidel Martinez-Gutierrez reported that 24 nm of AgNPs has dose-dependent cytotoxic effects. Concentrations ≤ 6.25 µg/ml of AgNPs had minimal cytotoxic effects on macrophages. Whereas, concentrations ≥ 12.5 µg/ml exhibited significant cytotoxic effects and almost killed 50% of macrophages (33). In the present study, we used AgNPs along with L-arginine to increase the antibacterial effect of AgNPs and reduce their toxicity. L-arginine is a vital amino acid for wound healing and fracture healing (43, 44). The peptides rich in arginine have high antimicrobial activities, which is due to its interaction with bacterial cell wall (45, 46). A few studies have evaluated the antibacterial effect of L-arginine (23, 47, 48). Deepa et al. evaluated the antibacterial effects of L-arginine/L-tryptophan, D-arginine/D-tryptophan and L-leucien/L-arginine on several wound-associated bacteria, and reported that all three dipeptides have antibacterial properties and synergistic effects when combined with ampicillin (47). In our study, L-arginine had antibacterial effects at concentration of 25 mg/ml, but addition of the MIC and MIC50 concentrations of L-arginine increased the antibacterial effect of AgNPs. The MIC

of AgNPs with L-arginine were 3.9 and 1.9 µg/ml. Therefore, it can be concluded that L-arginine had synergistic effects on AgNPs. Furthermore, L-arginine is a non-enzymatic antioxidant that can reduce the toxic effects of nanoparticles (49). Takayama et al. reported that L-arginine-silvernitrate had antibacterial effects against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *P. aeruginosa* (23), which is consistent with our study. Tang et al. also reported that L-arginine-chitosan had significant inhibitory effect on *P. fluorescens* and *E. coli* (50). Further studies should be performed to examine the toxic effects of AgNPs and L-arginine on human cells in vivo.

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CONCLUSION

Our study suggests that AgNPs along with L-arginine can be used as an alternative antibacterial agent against *P. aeruginosa*, and might be useful for treatment of wound infections.

ACKNOWLEDGMENTS

The authors acknowledge the Urmia University for their financial support in this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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