RESEARCH PAPER

Enhanced effect of Amikacin in conjugation with gold nanopartcles as a carrier to kill *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa is a significant cause of nosocomial infections. Infection caused by this organism is difficult to treat due to the presence of its innate resistance to many antibiotics. Conjugation of antimicrobial drugs with nanoparticles has emerged as an innovative and promising alternative that enhances therapeutic effectiveness. The current study focuses on the use of gold nanoparticles (GNPs) as a carrier to enhance the antibacterial effect of amikacin against *P. aeruginosa*. Amikacin was conjugated with GNPs. The success of conjugation was studied by UV/Vis and FTIR spectroscopies. The labeling efficiency was measured by HPLC and atomic absorption spectrometer analyses. The antibacterial activity of amikacin-GNPs conjugate was investigated by disc diffusion and liquid broth dilution methods. The labeling efficiency showed that 450 amikacin molecules were attached to each GNP. The zone of growth inhibition of amikacin-GNPs complex and amikacin by disc diffusion method were 37± 0.118 and 35± 0.149 mm. Minimum inhibitory concentration (MIC) of the conjugate and amikacin alone were determined 23.43 and 46.87, mg/ml, respectively. Minimum bactericidal concentration (MBC) of the conjugate and amikacin alone were determined 46.87 and 93.75 mg/ml, respectively. The results showed the enhanced antibacterial effect of amikacin-GNPs complex in comparison with amikacin alone against *P. aeruginosa.*

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INTRODUCTION

During last few decades, there has been a dramatic increase in the emergence of antibiotic-resistant bacteria, resulting in elevated bacterial pathogenesis at the worldwide level [1, 2].

The extensive use of antibiotics in the hospitals and community has fueled this crisis. Consequently, bacteria such as *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococci*, and *S. aureus*, and some members of the *Pseudomonas* and Enterobacteriaceae families, are now resistant to nearly all the conventional antibiotics [3]. In particular, the emergence of drug resistant strains of *P.aeruginosa* infections, indicates that drug resistance is a major problem to public health [1, 2]. The resistance mechanism of the bacteria, such as reduced outer membrane permeability and efflux of antibiotic drug, is mainly associated with its physical properties viz. size, charge, or intrinsic property of the molecule [4].

For a successful antibiotic therapy, the dose should be diminished to avoid their side effects [5-7]. Recently, there has been a considerable research interest in the area of nanoparticle-based drug delivery systems as carriers for various small and

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large molecules [8]. Nanoparticles provide versatile platforms for therapeutic applications based on their physical properties [9]. Among the different nanoparticles, GNPs are well-suited for a wide range of biological applications because of well-developed surface chemistry, small size, less toxicity and stability into the cells [10]. Large surface to volume ratio of GNPs offer a large number of drug molecules being carried by the GNPs [7]. Also, GNPs have shown to increase drug concentration at infected site with reduced toxicity of the drug [11].

Based on the recent research efforts, conjugation of drugs with nanoparticles can enhance the antimicrobial activity of the drugs [12]. In this regard, Enayatimoghaddam et al. evaluated the antimicrobial effect of GNPs in combination with vancomycin on the growth of isolates resistant to vancomycin. Their results showed good antibacterial effects of GNPs in combination with vancomycin in high densities against all the drug-resistant *enterococci* strains [13].

In this work, we used amikain in conjugation with GNPs in spherical shape against *P. aeruginosa.* Amikacin was selected as a proper antibiotic because it is most often used for treating severe hospital-acquired infections with multi drug resistant Gram-negative bacteria. Also, the existence of four NH_2 groups in chemical structure of amikacin causes its strong binding to GNPs.

MATERIALS AND METHOD

Materials

GNPs 10-12 nm and amikacin sulfate were purchased from Sigma Aldrich, USA. Nutrient agar and nutrient broth media were purchased from Merck, Germany. *Pseudomonas aeruginosa* strain ATCC 27853 was purchased from Pasteur Institute, Tehran, Iran.

Preparation of amikacin-GNPs conjugate

In order to conjugating, 10 ml of gold nanoparticles (size: 10-12 nm; spherical shape; concentration: 100 ppm) were added dropwise to the dilute amikacin (6 mg/ml) and was stirred for 15 min. The solution was incubated at room temperature for 24 h and stored in refrigerator for further studies [14].

Characterization of amikacin-GNPs conjugate

The formation of amikacin–GNPs conjugate was monitored by UV–Vis spectrometer (Cary 100 Bio, Varian, USA). The gold nanoparticles and amikacin exhibit plasmon resonance peaks at 520 and 280 nm, respectively [15, 16].

FTIR spectrum of the sample was recorded by Perkin-Elmer Fourier transform infrared spectroscopy (Nicolet Magna 560 IR, USA) to demonstrate attachment of amikacin to GNPs [15, 17].

Determination of the labeling efficiency

The concentration of amikacin and GNPs at the conjugate was measured by HPLC (high-performance liquid chromatography) and atomic absorption spectroscopy, respectively. Then, the concentration of amikacin was divided on the concentration of GNPs [18, 19].

To determine the amount of free amikacin, 10 ml of the sample was centrifuged at 13500 rpm for 10 min. Then, the supernatant was injected into the HPLC system ((Waters, model 2478, Arcade, NY) [6, 20].

The concentration of GNPs was determined by ashing the samples with addition of sulfuric acid at 300–350°C. Then, 3 ml hydrochloric and 1 ml nitric acids were added to the ash. The sample was dissolved in 0.5 N hydrochloric acid and was analyzed by an atomic absorption spectroscopy (model AA240FS, VARIAN, CA) [18, 15].

Antibacterial activity of amikacin-GNPs complex

This assay was investigated by a disc diffusion method. A suspension of *P. aeruginosa* (1.5×10^{8} CFU/ml) was grown on nutrient agar. The discs made from filter paper, were placed on the agar plates and then 10 µl amikacin and 10 µl amikacin-GNPs complex were put on the discs. Also, only GNPs were used as negative control. The plates were incubated at 37°C for 24 h. After incubation, the zone of inhibition in millimeter around each disc was measured [16].

MIC and MBC

A broth macrodilution method was used for determination of MIC and MBC. The different concentrations of amikacin–GNPs conjugate, GNPs (0.1 mg/ml) and free amikacin (6 mg/ml) were prepared in nutrient broth. The inoculum (1 ml) was adjusted to yield a concentration of 1.5×10^{8} CFU/ml of *P. aeruginosa* suspension. The tubes were incubated at 37 °C for 16 h, while a medium without amikacin or amikacin-GNPs conjugate was used as control. The MIC was the lowest concentration of amikacin and conjugate that

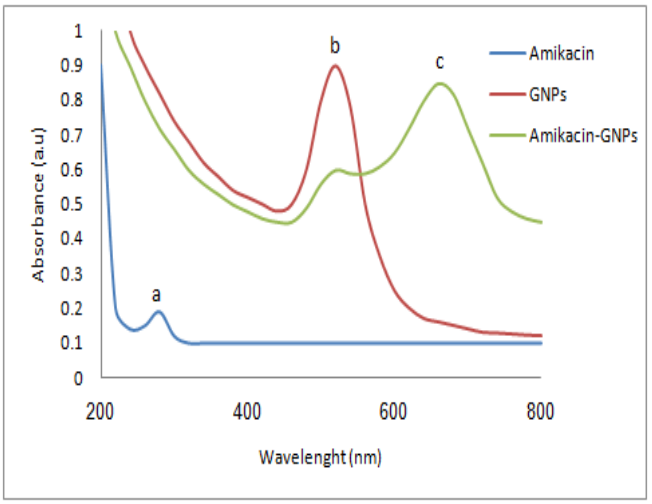

Fig. 1. UV–vis spectra of (a) amikacin)b) GNPs and (c) amikacin-GNPs conjugate.

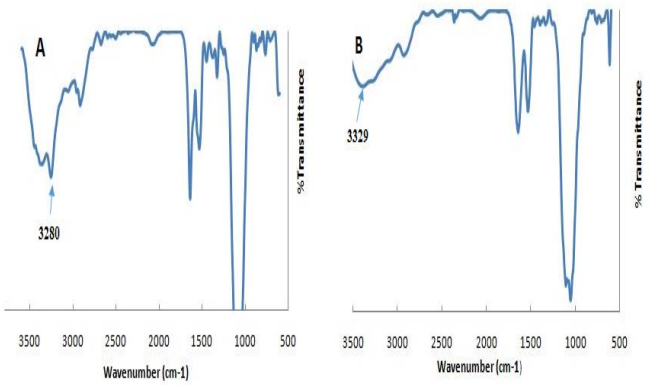

Fig. 2. Infrared spectra of amikacin (A); and amikacin-GNPs conjugate (B).

could inhibit the bacterial growth. The MBC was the lowest concentration of amikacin and conjugate that could kill 99.9 percent of bacteria [3, 6, 21].

RESULTS AND DISCUSSION

UV-vis spectroscopy

The UV–Vis spectroscopy showed the binding of amikacin to GNPs. The initial GNPs and amikacin had Plasmon resonance near 520 and 280 nm, respectively. After conjugation of amikacin with GNPs, the appearance of a new peak at 660 nm approved the production of amikacin-GNPs conjugate (Fig.1).

FT-IR spectroscopy

The peak at 3280 cm^{-1} is attributed to the amino groups. After the conjugation process, the appearance of the characteristic peak of the NH_2 stretching at 3329 cm^{-1}demonstrated direct binding of amikacin molecules to GNPs (Fig. 2).

A schematic is shown in Fig. 3 to illustrate the conjugation process. GNPs reacted with amikacin molecule by ionic interaction between amino group of amikacin and negative surface charge of GNPs.

Gold nanoparticles were capped with citrate and the surface of nanoparticles was negatively charged. On the other, amikacin has four NH_2 groups and has a positive charge on its surface. GNPs reacted with amikacin molecule by ionic interaction between amino group of amikacin and negative surface charge of GNPs.

HPLC analysis

The HPLC analysis determined that 82±3/4%

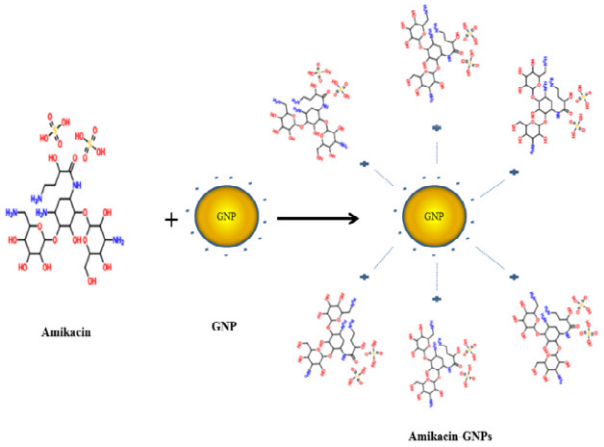

Fig. 3. A schematic representation of GNPs–amikacin conjugation.

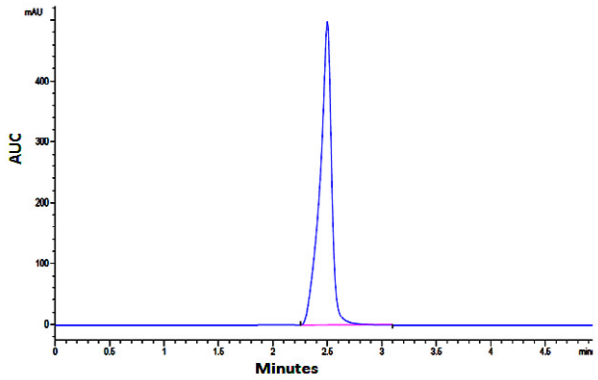

Fig. 4. Representative HPLC chromatograms of amikacin conjugated to the GNPs.

Table 1. Means of zone of growth inhibition (mm) of amikacin and a amikacin–GNPs complex on *P. aeruginosa*

Amikacin concentration (600 ppm) / GNPs concentration (100 ppm)	Inhibition zone diameter (mm)
Amikacin	35± 0.149
Amikacin-GNPs	37± 0.118
GNPs (control)	0

of amikacin molecules were attached to the GNPs (Fig.4).

The concentration of GNPs at the conjugate measured by atomic absorption spectroscopy was 75±1.4%.The calculations showed that 450 amikacin molecules were attached to each GNP.

Microbial efficacies of amikacin-GNPs complex

The growth inhibition affected by amikacin-GNPs complex against *P. aeruginosa*is is given in Table 1. There were no significant differences in antibacterial activity between pure amikacin and amikacin-GNPs complex. In a similar study,

Table 2. MIC and MBC of amikacin and amikacin-GNPs conjugate against *P. aeruginosa*.

Sample	MIC (ppm)	MBC (ppm)
Amikacin	46.87	93.75
Amikacin-GNPs	23.43	46.87

Burygin et al. explored the antibacterial activity of a mixture of gentamicin and colloidal-gold particles against *E. coli*, using the agar-well-diffusion method. Their results showed no differences between the antibacterial activity of gentamicin and that of a gentamicin–GNPs mixture [15]. GNPs themselves do not have any antimicrobial activity, they may act as drug carriers. In other words, because of the presence of GNPs, the surface area increases and hence it carries more drugs on its surface [15].

MIC and MBC of amikacin-GNPs complex

According to Table 2 MIC and MBC of the GNPs conjugated with amikacin were lower than free amikacin. We observed turbidity in all the tubes including different concentrations of GNPs. These findings showed excellent antibacterial activity of amikacin-GNPs conjugate in comparison with free amikacin.

Similarly, Ali et al. reported antibacterial activity of silver and gold nanoparticles conjugated with cefixime against *Staphylococcus aureus*. Their results confirmed that the bactericidal potential of cefixime was enhanced by 8 and 3 times upon conjugation with gold and silver nanoparticles respectively [3].

The results indicated that GNPs-amikacin conjugate is more efficient against *P. aeruginosa* than free amikacin [16]. In previous study, Ahangari et al. investigated the antibacterial effect of gold nanospheres conjugated with gentamicin against *S.aureus* [18]. In another study in nanobiotechnology research center of Zanjan university, Salouti et al. used gentamicin conjugated with gold nanorods against *Staphylococcus aureus*. Their results showed that GNPs in both rod and sphere shapes can enhance the antibacterial activity of gentamicin against *S. aureus* in comparison with free antibiotic [6, 22]. Similarly, Chavan et al. investigated the antibacterial activity of ampicillin conjugated with gold nanoparticles against ampicillin resistance *Escherichia coli* bacteria. Their results indicated that the efficacy of ampicillin conjugated with gold nanoparticles increased against ampicillin resistance bacteria [4]. Likewise, these studies suggests that amikacin conjugated with GNPs presumably retains its activity because GNPs alone were nontoxic, whereas GNPs-amikacin conjugate became a potent bactericide effective against *P. aeruginosa* which were unaffected in media containing high levels of soluble amikacin alone [16]. The mechanism of interaction between nanoparticles and microorganisms has been ascribed to their ability to penetrate the cell wall causing structural changes, degradation and eventually cell death [16]. The large surface area is a significant feature of GNPs, so they are able to carry numerous antibiotics in to the infection foci. Finally, nanotechnology-driven approach to treating antibiotic resistant bacteria is feasible and needs to be developed and tested in *in vivo* model systems.

CONCLUSION

This study showed the significant enhancement of antibacterial activity of amikacin in conjugation with GNPs against *P.aeruginosa* in comparison with amikacin alone.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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