The antibacterial effect of silver nanoparticles on some bacterial pathogens

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Abstract

Nanoparticles are a special group of materials with unique features and extensive applications in diverse fields. The use of nanoparticles of some metals is a viable solution to stop infectious diseases due to the antimicrobial properties of these nanoparticles. The present work demonstrates the effect of silver nanoparticles (AgNPs) on the antibacterial activity of four different antibiotics (amoxicillin, ceftriaxone, chloramphenicol, and penicillin) against eleven Gram-positive and Gram-negative isolates. Disk diffusion method was used to determine the antibacterial activity of various classes of antibiotics in the absence and presence of sub-inhibitory silver nanoparticles of concentration (80 microgram/ml). A synergistic effect was observed between AgNPs and both amoxicillin and penicillin for Gram-positive isolates and between AgNPs and both ceftriaxone and chloramphenicol for Gram-negative isolates.

Key words

Silver ion nanoparticles, antimicrobial, inhibition zone.

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التأثير الضد ميكروبي للاجسام النانوية للفضة على بعض الممرضات البكتيرية

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فرع العلوم الاساسية ، كلية طب الاسنان ، جامعة بغداد ، بغداد ، العراق

الخلاصة

ان الاجسام النانوية هي مجموعة خاصة من المواد ذات مواصفات فريدة وتطبيقات كبيرة في مختلف المجالات. لقد وجد ان استعمال الاجسام النانوية لبعض المعادن هو الحل الامثل لايقاف انتقال الامراض المعدية وذلك يعود الى الصفات الضد ميكروبيه لهذه الاجسام النانوية. لقد اوضحت الدراسة الحالية تأثير استخدام الاجسام النانوية للفضة على الفعالية الضد ميكروبيه للمضادات الاربعة (الاموكسيسيلين، السيفترايكسون، الكلورامفينيكول والبنسلين) ضد احدى عشر عزلة من البكتريا الموجبة والسالبة لصبغة غرام وقد استخدمت تقنية الانتشار من الاقراص لتحديد الفعالية الضد ميكروبيه لهذه المضادات المختلفة في وجود وغياب التركيز التحت التثبيطي (80مايكروغرام/مللتر) لمحلول الاجسام النانوية للفضة. لقد لوحظ تأثير ايجابي فعال عند استعمال محلول الاجسام النانوية للفضة مع كل من الاموكسيسيلين والبنسلين ضد البكتريا الموجبة لصبغة غرام وبين نفس المحلول وكل من السيفترايكسون والكلورامفينيكول للبكتريا السالبة لصبغة غرام.

Introduction

Elemental silver and silver salts have been used for decades as antimicrobial agents in curative and preventive health care [1].

Recent studies have demonstrated that specially formulated metal oxide nanoparticles have good antimicrobial activity [2]. The antibacterial activity of the synthesized silver particles has

been investigated against Salmonella typhi, Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa, E.coli, Klebsiella pneumonia [3]. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution[4].

Silver ions work against bacteria in a number of ways; silver ions may interact with the thiol groups of enzyme and proteins that are important for the bacterial respiration and the transport of important substance across the cell membrane and within the cell [5]; silver ions may bind to the bacterial cell wall and thus, altering the function of the bacterial cell membrane [6]. Furthermore, Silver can inhibit enzymatic systems of the respiratory chain and alter DNA (deoxyribonucleic acid) synthesis [7, 8]. Thus silver metal and its compounds are the effective in preventing bacterial infection of wounds [9].

Microbes are unlikely to develop resistance against silver, as they do against conventional and narrow target antibiotics because the metal attacks a broad range of targets in the organisms which means that they would have to develop a host mutation simultaneously to protect themselves. Thus silver ions have been used in dental resin composites [10], in synthetic zeolites [11] and in coatings of medical devices [12].

The Silver nanoparticles have some advantage over silver salts because they are more stable against dissolution and diffusion to the surface of the materials to be protected. Silver salts and silver complexes have been studied in detail, but the antimicrobial activities of silver ions and silver ion-containing Ag nanoparticles have not been reported yet [13].

The objective of this study was to compare the antibacterial effect of silver solution with four common antibiotics (amoxicillin, chloramphenicol, ceftriaxone and penicilliin) using various microbial isolates and to show the antibacterial effect of combination of this solution with the four antibiotics on the same bacterial isolates. Such a comparative study would reveal isolate specificities and would eventually lead to better utilization of nanoparticles for specific application. Six representative bacteria (eleven isolates), typically recommended for use in the current antimicrobial assays comprising: Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, E.coli, Pseudomonas spp. and Acinetobacter spp. The antimicrobial effect was quantified based on the inhibition zone measured by the disk diffusion test.

Materials and Methods

Synthesis of silver nanoparticles

Silver nanoparticles were synthesized by pulsed laser ablation of silver plate immersed in double distilled water (DDW). The silver target (purity of 99.99% provided from Merck India co.) was fixed at the bottom of a glass vessel containing 1 ml DDW. Ablation was carried out using focused output of pulsed Nd:YAG laser (type HUAFEI) of 1064 nm at a 32 J/cm^2 fluence operating with a repetition rate of 10 Hz per second and pulse width of 10 ns.. The laser beam was focused on the silver target using convex lens of 11 cm focal length. The optical absorbance spectrum of the produced nanoparticles was recorded using spectrophotometer. The nanoparticles concentrations were estimated by atomic absoprtion spectroscopy AAS, to be about 80µg/ml.

Antibacterial assay

The antimicrobial susceptibility of silver nanoparticles (80µg/ml) synthesized was investigated in comparison with four types of antibiotics (amoxicillin 25 µg, ceftriaxon 30 µg , chloramphenicol 30 µg, and penicillin 10 International unit(IU) that are commonly used in treatment of both Gram-negative and Gram-positive bacterial pathogens. The disk diffusion methods were used as antimicrobial susceptibility testing method. Disposable plates containing Muller-Hinton agar inoculated with eleven tested Gram-positive and Gram-negative bacteria were obtained from AL-Yarmook Hospital and Central Teaching Laboratories included three representative Gram-positive bacteria: Staphylococcus aureus (2), Staphylococcus epidermidis (2), Enterococcus faecalis (2) and three representative Gram-negative bacteria: E.coli (2), Pseudomonas spp.(2) and Acinetobacter spp.(1), at a concentration of 10^8 cfu/ml. Zones of inhibition were measured after 24 hr of incubation at 35-37ºC. The comparative susceptibility of discs containing amoxicillin, chloramphenicol, ceftriaxone and penicillin impregnated with 30 µl of AgNPs was studied.

Statistical analysis

The mean and standard deviation (SD) reported for Ag nanoparticles and antibiotics with each microbial isolates were based on three replicates.

Results and Discussion

Figure (1) shows the SPE spectrum of the silver nanoparticles solution. It displays a quasisymmetric absorption band centred at 400 nm, which indicates that the nanoparticles in the growth solution are quasispherical approximately 8 nm in diameter. The silver nanoparticles solution was faint yellow in color [14-16].

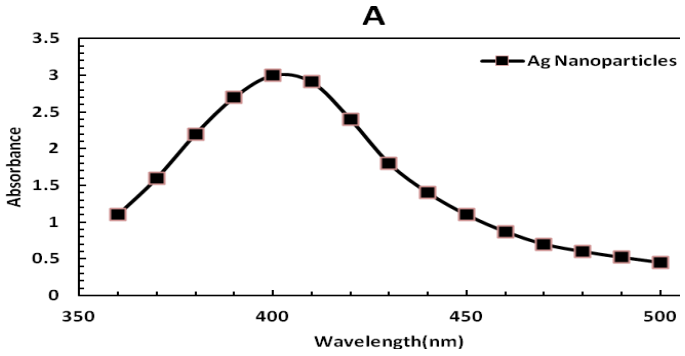

Fig.1: Absorption spectrum of Ag nanoparticles produced by laser ablation of 1064 nm and 32 J/cm^2 fluence in pure water.

In the present study the effect of Ag Nanoparticles on the antibacterial properties of different antibiotics (amoxicillin, ceftriaxone, chloramphenicol and penicillin) was investigated against six different species of bacterial pathogens : Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, E.coli, Pseudomonas spp. and Acinetobacter spp. (11 isolates) using the disk diffusion method.

Inhibition zone values were obtained for the synthesized AgNPs tested against those pathogens. The 80µg/ml AgNPs did not show an observed antibacterial activity against most of the studied pathogens whereas the inhibition zones were ranged from only 1-2 millimeter for the rest bacterial pathogens and this may be due to the low concentration of the

solution used which is considered as a sub-inhibitory concentration in this study. The results are presented as average values in Table (1) and as images in Fig.2.Table (1) shows the different sensitivities of the eleven bacterial isolates against the four antibiotics (amoxicillin, ceftriaxone, chloramphenicol and penicillin) used in this investigation. It shows that S.aureus 2 and Pseudomonas 2 are the most sensitive pathogens to ceftriaxone (CRO) with a diameter of inhibition zone ranges between 23-35mm. All the bacterial pathogens except Pseudomonas 1 show sensitivity to chloramphenicol (C). Most of the Gram-positive isolates show sensitivity to penicillin (P) and amoxicillin (AX), while most of the Gram-negative isolates show resistance to both antibiotics.

These results show a synergism between AgNPs and both P and AX for Gram-positive isolates and AgNPs and both CRO and C for Gram-negative isolates in a range starts from 1mm and not exceeded 14 mm. Other combinations show no effect or antagonism relationship for the rest tested isolates (Table 2). But, Sadeghi, et.al. (2010) showed that silver nanoparticles exhibited more activity than that showed in this study [17]. This may be explained by the low concentration of Ag-NPs (80µg/ml) used in this work. This fact was also proved by Parameswari, et.al. (2010) who showed that the inhibition zone increased as the concentration of $AgNO_3$ and AgNPs increased [18].

Fig.2: Inhibition zones of the four antibiotics (amoxicillin, ceftriaxone, chloramphenicol and penicillin) against E. faecalis in presence and absence of 80µg/ ml of silver nanoparticles.

Table (1): The comparative activities of various antibiotics with and without silver nanoparticles against eleven bacterial pathogens.

Bacterial pathogen	***Antibiotic type***							
	Mean inhibition zone in mm							
	CRO*	***C****	***P****	***AX****	***CRO****	***C****	***P****	***AX****
S.aureus 1	22±1	22±2	16±1	13±0	13±2	19±1	18±2	13±1
S.aureus 2	23±1	27±2	16±2	12±1	20±3	27±1	19±0	16±1
S.epidermidis 1	12±2	35±1	21±2	17±1	11±2	31±1	21±3	17±2
S.epidermidis 2	0	10±3	0	0	0	10±1	8±2	12±2
E.faecalis 1	19±3	25±1	16±3	8±1	13±2	9±3	18±1	10±2
E.faecalis 2	12±2	32±1	17±0	21±0	12±1	25±1	19±0	23±2
Pseudomonas 1	0	0	0	0	0	7±1	0	0
Pseudomonas 2	35±1	17±1	0	0	36±1	18±2	0	0
E.coli 1	0	22±1	7±2	0	0	30±0	7±1	0
E.coli 2	0	18±3	0	0	0	21±0	0	0
Acinetobacter spp.	8±1	10±0	0	0	8±1	24±1	0	0

*antibiotic alone, ** antibiotic combined with AgNPs

Table 2: The overall effect of combination of AgNPs and the four antibiotics on Diameter of inhibition zone (DIZ) of the eleven bacterial isolates.

Antibiotic	*Gram-positive isolates*	*Gram-negative isolates*
CRO	0,-	0,+
C	-	+
P	+	0
Ax	+	0

(-) Antagonism, (+) Synergism, and (0) no effect

The mechanism of inhibitory action of silver ions on microorganism shows that upon Ag+ treatment, DNA loses its replication ability and expression of ribosomal subunit proteins, as well as other cellular proteins and enzymes essential to ATP (Adenosine Tri-Phosphate) production, becomes inactivated [19]. It has also been hypothesized that Ag+ primarily affects the function of membrane bound enzymes in the respiratory chain. However, the mechanism of bactericidal actions of silver nanoparticles is still not well understood. The positive charge on Ag+ is an important factor for its antibacterial nature, through electrostatic interaction between the negatively charged cell membrane of the microorganisms and positively charged nanoparticles. It is proposed that the electrostatic force might be an additional cause for the interaction of the nanoparticles with the bacteria [20]. In a previous report [21] on the bactericidal activity of silver nanoparticles, it was shown that the interaction between silver nanoparticles and constituents of the bacterial membrane caused structural changes and damage to membranes, finally leading to cell death. A low antibacterial activity of AgNPs compounds may due either to decrease in particle size of silver nanoparticles that show a lower susceptibility or to low concentration of those compounds [22]. Previously reported studies showed that silver ions had effective antimicrobial properties at concentrations of 1ppm or less [23].

The extent of inhibition depends on the concentration of the silver nanoparticles as well as on the initial bacterial population [18]. This was supported by Sondi and Salopek-Sondi (2004) who reported that the interaction of these particles with intracellular substances from lyses cells caused their coagulation and the particles were thrown out of the liquid system [24]. Furthermore, Singh *et.al* (2008) reported that antibacterial effect was size and dose dependent and was more pronounced against Gram negative bacteria than Gram positive bacteria [25].

Although most studies have utilized spherical particles, truncated tri-angular shaped particles are reported to have greater bactericidal effect compared to that of spherical and rod-shaped particles [21]. It is also reported that bactericidal efficiency is affected by the type of microorganism. Additionally Sadeghi, *et.al*. (2010) clearly demonstrated that the antimicrobial activity is only due to nanosilver shapes impregnated inside the bacteria and not due to the individual bacteria [17]. The mechanism for microbial growth inhibition observed for silver is not entirely understood. Possible mechanisms involve the interaction of silver ions with biological macromolecules through proteins thiol groups (-SH). Monovalent silver ions (Ag^{+}) would replace (H^{+}) ions of

sulfhydryl or thiol groups, inactivating the protein, decreasing membrane permeability, and eventually causing cellular death. The reaction of monovalent silver with sulfhydryl groups produces a much more stable -S-Ag group only on the bacterial cell surface. Silver ions may not be capable of permeating through cell membranes to react with the interior -S-H groups, rendering silver relatively nontoxic to humans and animals [26].

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