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Research Article

Promising Antibacterial Effect of Copper Oxide Nanoparticles against Several Multidrug Resistant Uropathogens

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ABSTRACT

Background: Recently, nanotechnology has been demonstrated to be a promising application to overcome the problem of antibiotic resistance. In the present study, we aimed to determine the antibacterial activity of copper oxide nanoparticles (CuO NPs) on several multiple-drug resistant (MDR) uropathogenic strains.

Methods: This *in vitro* case-control study was performed on 4 uropathogenic bacteria including *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The antibacterial property was evaluated by well diffusion method at different concentrations of CuO NPs.

Results: Overall, NPs concentration of 10, 25 and 50 µg/mL showed the remarkable antibacterial activity. A lower effect was seen against *S. aureus* strains. CuO NPs exhibited maximum bacterial growth inhibition against *E. faecalis* strains. In most of the cases, the zone of inhibition in 50 µg/mL concentration was closest to control positive antibiotics.

Conclusion: In summary, CuO NPs as an alternative to conventional antibiotics that are currently used showed dose-dependent on antibacterial activity against different uropathogens, specificity towards pathogenic Gram-positive bacteria. This promising antibacterial activity of CuO NPs suggesting the development of NPs coatings on the different surface of biomedical materials for applications in different antimicrobial control systems.

Introduction

Nosocomial infections (NIs) are a common consequence of hospitalization which is associated with high morbidity and mortality.[1] Bacterial infections are one of the main causes of NIs and the frequently isolated pathogens are *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and enterococci.[2] Antimicrobial resistance increase is the major concern in hospital environments.[3] NIs are associated with longest hospitalization time and subsequently risk of emergence of resistant bacteria against available antibiotic agents.[1]

The profound effect of antimicrobial agents on the course of NIs is undeniable; however, with their broad use and abuse, the emergence of drug-resistant bacteria has become a major problem.[4] Previously, several studies reported the emergence of multiple-drug resistant (MDR) pathogens including vancomycin-resistant enterococci (VRE), methicillin-resistant *S. aureus* (MRSA) and extended spectrum β-lactamase (ESBL) producing Gram-negative bacteria among Iranian patients.[5-7]

Dealing with antibiotic-resistant bacteria is costly and requires more time and may be associated with side effects and sometimes therapeutic failure.[3,8] Therefore, researchers are looking for an alternative and novel antimicrobial agents.[9] In this case, nanotechnology has been demonstrated to be a promising application to overcome the problem of antibiotic resistance.[10] Recent studies showed that nanoparticles (NPs) can have a broad spectrum of antimicrobial activity against different clinically isolated bacteria.[10] Generally, the antimicrobial activity of the NPs may be varied based on their composition and type of pathogens.[10,11] Moreover, antimicrobial NPs exhibit numerous distinct benefits in reducing acute toxicity, overcoming the existing antibiotic resistance mechanisms, targeted drug delivery, combinatorial antibiotic delivery, and vaccine development when compared to conventional antibiotics.[12]

Compared to published reports on antimicrobial properties of metal NPs, very limited information is available on the antibacterial activity of NPs on clinically obtained isolates. In the present study, we aimed to

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determine antibacterial activity of CuO NPs on several MDR uropathogenic strains obtained from Iranian patients.

Materials and Methods

Study design and bacterial strains

This *in vitro* case-control study was performed in the Department of Bacteriology and Virology, Shiraz University of Medical Sciences, Shiraz, Iran in 2016. The bacterial strains used in the study were *S. aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and 10 clinical isolates from each mentioned bacteria. Clinical isolates were 40 nonduplicate bacteria obtained from urine specimens which were collected within 2012-2016 as parts of previous published and unpublished works from Nemazee hospital in Shiraz, Iran.[13,14] All clinical isolates were MDR according to previous published criteria.[15]

Characterization of nanoparticles

The used Copper oxide (CuO) NPs purchased from Neutrino Corporation, Iran. The proper amount of the nano CuO was added to distilled water and dispersed by sonication. After that, tween 20 was added to the solution and the stock solution was sonicated for 10 min. Four different concentrations (5, 10, 25, and 50 µg/mL) of nano CuO for antibacterial assay were provided.

Antimicrobial susceptibility testing

Antibacterial property of the CuO NPs against Gram-positive and negative bacteria was evaluated by well diffusion method on Muller-Hinton agar (MHA; Merck, Germany) described by Nanda et al.[16] Briefly, the bacterial suspension was prepared by making a saline suspension of bacterial colonies. The suspension was adjusted to the tube of 0.5 McFarland turbidity standard, which equals to 1.5×10^8 colony forming units (CFU)/ml. The suspension was inoculated on the surface of MHA by using a sterile swab. Then, 5 mm wells were punched into the nutrient agar plates for testing NPs antimicrobial activity. Finally, 30 µL of different CuO NPs concentration was added into the wells. All plates were incubated on a rotary shaker at 160 rpm at 37 °C for 24 h and the clear zone of the growth inhibition was measured. Moreover, antibiotic susceptibility was determined toward teicoplanin (30 µg), imipenem (10 µg), polymyxin B (300 units) and fosfomycin (200 µg) antibiotic discs (MAST, UK) as the positive control. All tests were done in triplicate.

Statistical analysis

Analysis was performed by using SPSS™ software, version 21.0 (IBM Corp., USA). The results are presented as descriptive statistics in terms of relative frequency. Values are expressed as the mean ± standard deviation (continuous variables) or percentages of the group (categorical variables). Chi-square or Fisher's exact tests was used to estimate the statistical association. Statistical significance was regarded as P values less than 0.05.

Results

The size and purity of the used spherical CuO NPs in the present study were < 50 nm and 99%, respectively (Figure 1). Antibacterial susceptibility testing revealed CuO NPs inhibited the growth of uropathogenic bacteria (Figure 2). The full results of antibacterial activity of CuO NPs against Gram-positive and negative uropathogenic bacteria are presented in Tables 1 and 2, respectively. Overall, NPs concentration of 10, 25 and 50 µg/mL showed remarkable antibacterial activity with a zone of inhibition more than 10 mm. A lower effect was seen against *S. aureus* strains, since none of the isolates was susceptible to 10 µg/mL concentration. Moreover, the most sensitive isolates toward CuO NPs were *E. faecalis* strains.

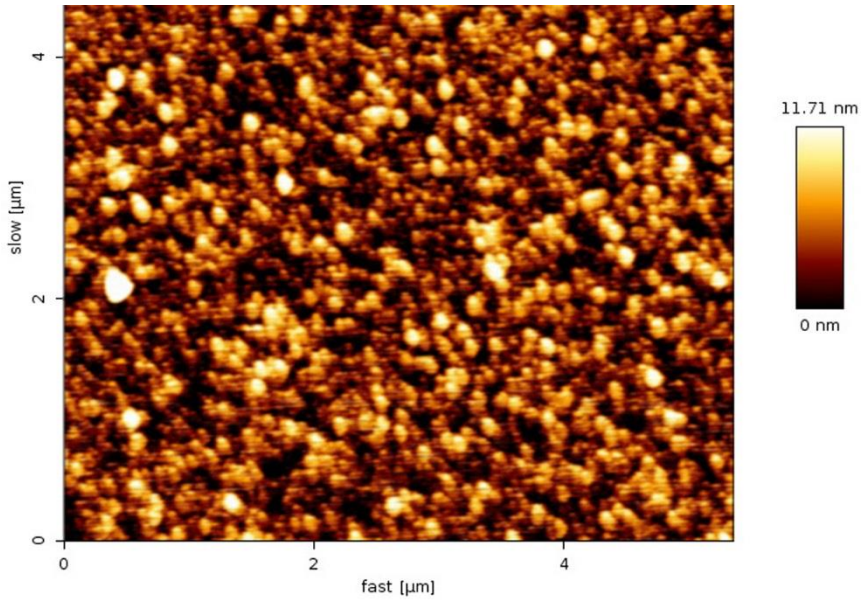

Figure 1. Atomic force microscopy (AFM) image of copper oxide (CuO) nanoparticles.

CuO NPs exhibited maximum bacterial growth inhibition against *E. faecalis* uropathogenic isolates and *E. faecalis* standard strains with zone of inhibition 22.9 ± 1.7 mm and 22 ± 1 mm, respectively. The majority of clinical isolates showed higher sensitivity to CuO NPs compared to standard strains. In most of the cases, the zone of inhibition in 50 µg/mL concentration was closest to positive control antibiotics.

Discussion

Urinary tract infections (UTIs) are the most common urological disease approximately known to occur in all age groups.[17] *E. coli*, *S. aureus*, *E. faecalis* and *P. aeruginosa* are prevalent uropathogenic bacteria which are well-known for biofilm producing ability.[17,18] Catheter-associated UTI accounts for a high rate of nosocomial infections mostly associated with biofilm producing bacteria.[18]

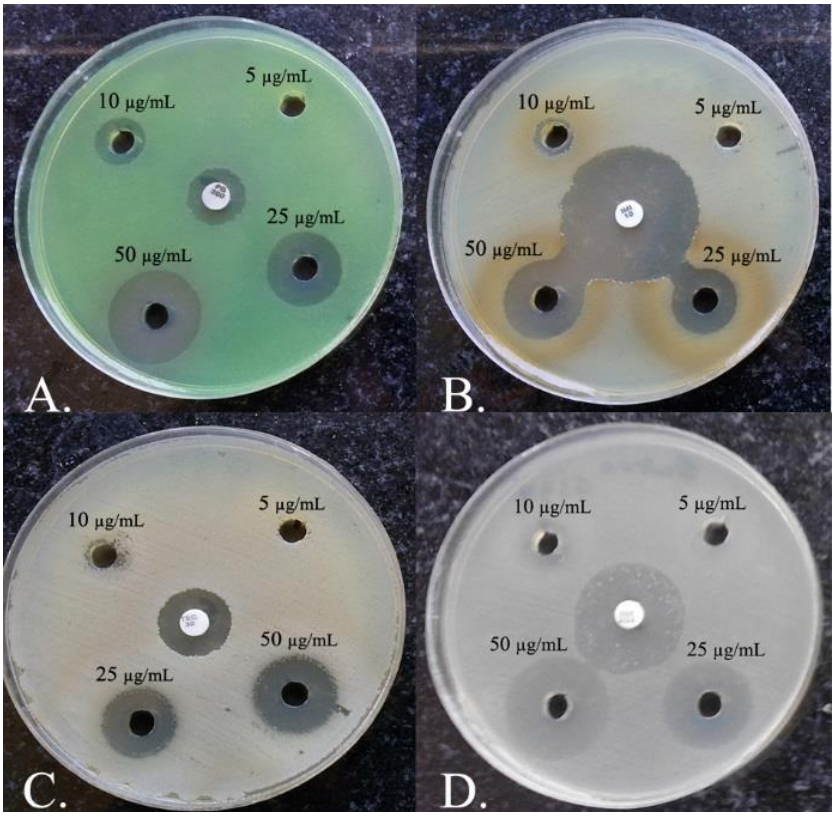

Figure 2. Antibacterial activity of copper oxide nanoparticles against uropathogens by well diffusion method; A, *P. aeruginosa*; B, *E. coli*; C, *S. aureus*, D, *E. faecalis*.

Table 1. The mean diameter (mm) zone of inhibition of Gram-positive strains against nanoparticles.

Concentration	ATCC 25923 (mean ± SD)	*Clinical S. aureus* (mean ± SD)	P value	ATCC 29212 (mean ± SD)	*Clinical E. faecalis* (mean ± SD)	P value
CuO 5 µg/mL	0	0	ND [a]	0	0	ND
CuO 10 µg/mL	0	0	ND	10 ± 1	9.4 ± 0.8	0.3
CuO 25 µg/mL	12.7 ± 0.6	14.4 ± 1.3	0.06	13.7 ± 1.1	14 ± 1.6	0.77
CuO 50 µg/mL	15.7 ± 0.6	17.4 ± 1.2	0.04	22 ± 1	22.9 ± 1.7	0.41
Teicoplanin (30 µg)	18.7 ± 0.6	14.7 ± 0.8	<0.001	-	-	-
Fosfomycin (200 µg)	-	-	-	26 ± 1	27.7 ± 3.4	0.42

[a]Not determined

Table 2. The mean diameter (mm) zone of inhibition of Gram-negative strains against nanoparticles.

Concentration	ATCC 25922 (mean ± SD)	*Clinical E. coli* (mean ± SD)	P value	ATCC 27853 (mean ± SD)	*Clinical P. aeruginosa* (mean ± SD)	P value
CuO 5 µg/mL	0	0	ND [a]	0	0	ND
CuO 10 µg/mL	9.3 ± 0.6	9.1 ± 0.6	0.62	10 ± 1	10.6 ± 0.8	0.31
CuO 25 µg/mL	13 ± 1	14 ± 1.2	0.22	14 ± 1	16.1 ± 1.1	0.01
CuO 50 µg/mL	15 ± 1	16.7 ± 1.4	0.08	18.3 ± 0.6	19.4 ± 1.2	0.16
Imipenem (10 µg)	30.6 ± 0.6	30.8 ± 1.1	0.77	-	-	-
Polymyxin B (300 units)	-	-	-	13 ± 1	13.5 ± 1.2	0.52

[a]Not determined

Resistant nature of biofilm community to antibiotics and host defenses eradication of causative agents are challenging to physicians.[18] Coated NPs catheters can be a promising solution and may be useful in reducing the biofilm formation risk in patients with indwelling catheters.[18] Since, it has been suggested that NPs due to their small size may directly penetrate through the exopolysaccharide structures of biofilm and deliver anti-microbial function.[12]

In the present study, CuO NPs showed promising antibacterial effects on several Gram-positive and negative strains. Previously, in accordance to our findings several studies documented antimicrobial properties of copper NPs on different bacterial strains including *E. coli*, *S. aureus* and *P. aeruginosa*.[19-21] NPs can induce their antibacterial effects through different mechanisms including rupture via attachment to the negatively charged bacterial cell wall, reacting to sulfhydryl groups and causing respiration blockage and cell death, proton motive force destruction or binding with DNA molecules and leading to helical disruption.[11,22]

In our results, NPs showed their antibacterial effects in a dose-dependent manner, since zone of inhibition increased with elevating concentration. In agreement with our finding two reports showed dose dependence antibacterial activity of CuO and silver (Ag) NPs on different Gram-positive and -negative pathogens.[20,23] The effective concentrations of NPs in the present study ranged from 10 to 50 µg/mL. Our results was comparable with previous findings in experiments with different metal oxide NPs;[11] however, biological activity of NPs may vary depending on the type of NPs, size, synthesis method, and the tested organism.[11,20]

It was previously documented that the Ag, CuO and ZnO NPs antibacterial effect is more significant against Gram-positive strains than Gram-negative strains.[24,25] As seen in our results, CuO NPs exhibited maximum growth suppression against Gram-positive strains (*E. faecalis* strains). The structural dissimilarities of the external cell wall of Gram-positive and Gram-negative bacteria may be an explanation for such observations.[24]

Several authors showed antimicrobial effects of smallest Cu NPs ranging from 8 nm to 40 nm, which was comparable with results of our tested CuO NPs with a mean size less than 50 nm.[26-28] Based on previous reports, NPs with different mean size can exhibit different antibacterial activity.[29] It has been proposed that metal NPs in suspension may release metal ions into the medium.[29] In this regard smaller NPs can facilitate faster dissolution and showing higher level of toxicity.[30] The combined effect between the activity of the NPs and releasing ions may result in cell disruption.[31]

As shown in the results, CuO NPs have interesting antibacterial effects; however, the literature has reported a decrease of cell viability in cell lines exposed to metal NPs.[32] As one of our limitations, it was probable that the cytotoxic effects of CuO NPs also evaluated. However, Prabhu et al. showed that in short-time period copper NPs at low concentrations (10 and 20 µM) did not any significant effect on cell culture morphology and cell viability.[33] Moreover, the lack of minimum inhibitory concentrations (MICs) evaluation can be mentioned as final limitation.

Conclusion

In summary, CuO NPs as an alternative to conventional antibiotics that are currently used showed dose-dependent antibacterial activity against different uropathogenic bacteria, specificity towards pathogenic Gram-positive bacteria. This promising antibacterial activity of CuO NPs suggesting the development of NPs coatings on the different surface of biomedical materials for applications in different antimicrobial control systems.

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Conflict of Interests

The authors claim that there is no conflict of interest.

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