In vitro antibacterial potential of metal oxide nanoparticles against antibiotic resistant bacterial pathogens

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Objective: To investigate the antibacterial potential of 5 different metal oxide nanoparticles against antibiotic resistant bacterial pathogens viz., Pseudomonas aeruginosa, Klebsiella sp. Streptococcus pneumoniae, Staphylococcus aureus and Streptococcus sp. Methods: The antibacterial activity of the five different nanoparticles was assessed by well diffusion method. Different concentrations of the nanoparticles were analyzed by MIC and MBC techniques. Finally the potential MgO nanoparticle was also subjected for the time kill assay method. Results: The results reveal that, the MgO nanoparticle showed maximum sensitivity [(16.00±0.53) mm dia] against Streptococcus pneumoniae and showed minimum sensitivity against Klebsiella sp. [9.00±0.31) mm dia]. None of the nanoparticles showed sensitivity against the Streptococcus sp. The MIC result reveals that, the MgO nanoparticle showed maximum inhibition at a concentration of 10 µg against Streptococcus pneumoniae. Moreover, the time kill assay reveals that, the bacterial growth was inhibited from the 2nd h onwards at a concentration of 10 µg. Conclusions: It is concluded from the present findings that, the MgO nanoparticle could be used as an alternative antibacterial agent after completing successful in vivo trials.

1. Introduction

The infectious diseases are one of the major health problems to the developing and developed countries. During the last decade, various resistant mechanisms have been increased worldwide in bacterial pathogens which lead to failure treatment in human and animal diseases[1,2]. Bacteria are able to adapt rapidly to new environmental conditions such as the presence of antimicrobial molecules and, as a consequence, resistance increases with the antimicrobial use[3-4]. Recently, the metal oxide nanoparticles played a vital role in the novel drug delivery systems[5]. Synthesis of noble nanoparticles has been used as an antibacterial agent, catalysis, environmental and biotechnology is an area of constant interest[6]. Moreover, the biosynthesized and chemically synthesized silver nanoparticles showed various biological activities[5,7,8]. However, studies related with the antibacterial agents from metal oxides nanoparticles against antibiotic resistant bacterial pathogens are poorly understood. In this connection, the present study is made an attempt to find out the antibacterial potential of metal oxide nanoparticles.

2. Materials and methods

Commercial nanoparticles of Al₂O₃, Fe₃O₄, CeO₂, ZrO and MgO were procured from Sigma Aldrich Company, India. The characteristics of the nanoparticles are presented in Table 1.

Table 1

| Formula | Molecular weight | Form        | Particle size in Transmission electron microscopy (nm) |
|---------|------------------|-------------|--------------------------------------------------------|
| Al₂O₃   | 101.96           | Powder      | <50                                                   |
| Fe₃O₄   | 231.53           | Powder      | 9–11                                                  |
| CeO₂    | 172.11           | Powder      | <25                                                   |
| ZrO₂    | 123.22           | Powder      | <100                                                  |
| MgO     | 40.30            | Powder      | <30                                                   |

2.1. Test organisms
Five antibiotic resistant pathogens viz., *Pseudomonas aeruginosa*, *Klebsiella* sp., *Staphylococcus aureus* and *Streptococcus pneumoniae* were obtained from Vinayaga Mission hospital, Salem, Tamil Nadu, India.

2.2. Antibacterial assay

The antibacterial activity of the chosen nanoparticles was performed by using well diffusion method. About 20 mL of sterile molten Mueller Hinton agar (Himedia Laboratories Pvt. Limited, Mumbai, India) was poured into the sterile petriplates. Triplicate plates were swabbed with the overnight culture (10^6 cells/mL) of chosen pathogenic bacteria. Then the solid medium was gently punctured with the help of cork borer to make a well. Finally, the nanoparticle samples (50 μg/mL) were added from the stock into each well and incubated for 24 h at (37±2)°C and the antibacterial sensitivity is measured as zone of inhibition in millimeter in diameter.

2.3. Minimum inhibitory concentration (MIC)

Different concentrations (10, 20, 30, 40, 50 and 60 μg/mL) of metal oxide nanoparticles were prepared with dimethyl sulphoxide (DMSO) and mixed with 450 μL of nutrient broth and 50 μL of 24 h old bacterial inoculum and allowed to grow overnight at 37°C for 48 h. Nutrient broth with pathogens alone was served as negative control. Whole setup in triplicate was incubated at 37°C for 24 h. The MIC was the lowest concentration of the synthetic compounds that did not permit any visible growth of bacteria during 24 h of incubation after inoculation examined on the basis of turbidity(5).

2.4 Minimum bactericidal concentration (MBC)

To avoid the possibility of misinterpretations due to the turbidity of insoluble compounds if any, the MBC was determined by sub-culturing the above (MIC) serial dilutions after 24 h in nutrient agar plates using 0.01 mL loop and incubated at 37°C for 24 h. MBC was regarded as the lowest concentration that prevents the growth of bacterial colony on this solid media(5).

2.5 Time kill assay

The potential nanoparticle (MgO) which showed maximum sensitivity against *Streptococcus pneumoniae* was further subjected for time kill assay. The inoculum of *Streptococcus pneumoniae* (50 μL) at the concentration of 10^6 cells/mL was mixed with 50 μL (contains 10 μg/mL) of chosen nanoparticles and the total volume was made up to 5 mL by using minimal medium (g/L) [Sucrose-10; K2HPO4-2.5; KH2PO4-2.5; (NH4)2HPO4-1; MgSO4.7H2O-0.20; FeSO4.7H2O-0.01; MnSO4.7H2O-0.007 and H2O-1 000 mL]. The negative control was maintained without the nanoparticles. The growth of the bacterial species was assessed at every 1 h interval by measuring the optical density at 600 nm by using spectrophotometer (Cyber UV-1, Mecasys Co Ltd)[9].

3. Results

Antibacterial activity of the metal oxide nanoparticles against chosen antibiotic resistant bacterial pathogens was investigated and represented in Table 2. It reveals that, all the nanoparticles showed antibacterial activity against *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*. Of these, MgO nanoparticle showed maximum sensitivity [16.00 ±0.53 mm dia] against *Streptococcus pneumoniae* and the ZrO2 nanoparticles showed maximum sensitivity [12.00±0.51 mm dia] against *Pseudomonas aeruginosa*. The CeO2 nanoparticles showed minimum sensitivity [7.00±0.38 mm dia] against *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* [9.00 ±0.36 mm dia] respectively. Moreover, all the nanoparticles except Al2O3 showed sensitivity against *Klebsiella* sp. However, none of the nanoparticles showed sensitivity against *Streptococcus pneumoniae* and the CeO2 nanoparticles showed no inhibition against all the tested pathogens (Table 3). The time kill assay reveals that, the MgO nanoparticle inhibits the bacterial growth from the 2nd h after treatment (Figure 1).

### Table 2

Antibacterial activity of nanoparticles against antibiotic resistant pathogens (mm).

| Name of the nanoparticles | *Pseudomonas aeruginosa* | *Klebsiella* sp. | *Streptococcus pneumoniae* | *Staphylococcus aureus* | *Streptococcus pneumoniae* |
|---------------------------|-------------------------|-----------------|---------------------------|------------------------|---------------------------|
| Al2O3                     | 11.00±0.15             | –               | 11.00±0.41                | –                      | –                         |
| Fe2O3                     | 8.00±0.25              | 10.00±0.22      | 13.00±0.12                | 11.00±0.19             | –                         |
| CeO2                      | 7.00±0.38              | 7.00±0.16       | 9.00±0.36                 | 9.00±0.35              | –                         |
| ZrO2                      | 12.00±0.51             | 9.00±0.25       | 10.00±0.63                | –                      | –                         |
| MgO                       | 8.00±0.52              | 9.00±0.31       | 16.00±0.53                | –                      | –                         |

− no activity.

### Table 3

MIC and MBC of nanoparticles against antibiotic resistant pathogens (μ g/mL).

| Name of the nanoparticles | *Pseudomonas aeruginosa* | *Klebsiella* sp. | *Streptococcus pneumoniae* | *Staphylococcus aureus* | *Streptococcus pneumoniae* |
|---------------------------|-------------------------|-----------------|---------------------------|------------------------|---------------------------|
| Al2O3                     | 50                      | 50              | 50                        | 50                     | 50                        |
| Fe2O3                     | –                       | –               | 50                        | 50                     | 50                        |
| CeO2                      | –                       | –               | –                         | –                      | –                         |
| ZrO2                      | 60                      | 60              | 60                        | 60                     | 60                        |
| MgO                       | –                       | –               | 10                        | 10                     | 10                        |

−− no activity.
4. Discussion

Antibiotic resistance is the biggest challenge to the medical field for the treatment of infectious diseases. The antimicrobial agents have been categorized according to their mechanism of action[10]. The resistant bacteria spread and infection problems occur not only in the healthcare institutions but in the communities also. The spread of resistant bacteria within the community posses’ obvious additional problems for health control[11]. Recently, nanoparticles particularly, Fe3O4, ZrO2 and MgO showed antibacterial activities against ophthalmic pathogens[5]. The results of the antibacterial activity of the present study reveal that, the MgO nanoparticles showed maximum antibacterial activity against Streptococcus pneumoniae at the concentration of 10 μg from the 2nd h onwards. Generally, the nanoparticles bind with the thiol (-SH) groups of protein that destroy the cell wall[12]. But in the case of resistance bacteria, the possible mechanism of activity is, the MgO nanoparticles might inhibit the production of β-lactamase enzyme which involved in the drug deactivation process or the nanoparticles block the efflux pump pathway which involved in the drug elimination process[10]. Likewise, the TiO2, CdO and silver nanoparticles showed excellent antibacterial activity against Escherichia coli and Staphylococcus aureus[13-16]. This mechanism creates the stress in the cell wall and which produces more lactate dehydrogenase enzymes and leads to damage the cell membrane and the severity depends upon the exposure time[17]. It is concluded from the present study that, the MgO nanoparticle could be used as an effective antibacterial agent for the management of antibiotic resistant bacterial diseases after completing the successful clinical trials.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to the authorities of Alagappa University for providing required facilities and also to University Grants Commission, New Delhi for financial assistance and the grant number is: F.No. 39–563/2010 (SR).

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