Oxidative-Stress-Mediated Antimicrobial Properties of Metal-Based Nanoparticles

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Nanotechnologies have provided opportunities for the use of nanomaterials as components in the development of antibacterial agents. Indeed, metal-based nanoparticles (NPs) show an effective role in targeting and killing bacteria via different mechanisms, such as attraction to the bacterial surface, destabilization of the bacterial cell wall and membrane, and the induction of a toxic mechanism mediated by a burst of oxidative stress. Considering the lack of new antimicrobial drugs with novel mechanisms of action, the induction of oxidative stress represents a valuable and powerful antimicrobial strategy to fight MDR bacteria. Consequently, it is of particular interest to determine and precisely characterize whether NPs are able to induce oxidative stress in such bacteria.

metal-based nanoparticles | oxidative stress | ROS | antibacterial | mechanism of action

1. Introduction

The World Health Organization (WHO) states that the indiscriminate use of antibiotics has facilitated increasing bacterial resistance, and this has become a serious public health problem worldwide. Microbes, mainly bacteria, are now frequently resistant to several antibiotics; consequently, therapeutic options become more and more limited, and, concomitantly, nosocomial infections more and more severe [1]. The situation is so critical that in 2017 WHO published a list of 12 resistant bacteria which represent a real threat to human health [1][2]. These bacteria are divided by WHO into three groups according to their emergency profile in relation to resistance to antibiotics. The first group is bacteria classified as a priority, also called “Critical Urgency” and includes: *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacteriaceae*. They are Gram-negative bacteria, carbapenem-resistant, and third-generation-cephalosporin resistant. The second group is “High Urgency” and includes: vancomycin-resistant *Enterococcus faecium*, methicillin- and vancomycin-resistant *Staphylococcus aureus*, clarithromycin-resistant *Helicobacter pylori*, fluoroquinolone-resistant *Campylobacter* spp. and *Salmonella* spp., and third-generation-cephalosporin- and fluoroquinolone-resistant *Neisseria gonorrhoeae*. The third group is “Medium Urgency” and includes: penicillin-non-susceptible *Streptococcus pneumoniae*, ampicillin-resistant *Haemophilus influenzae*, and fluoroquinolone-resistant *Shigella* spp. [1][2].

Moreover, researchers have indicated that bacterial resistance to antibiotics due to genetic modification is correlated mainly with antibiotic consumption, and abundant antibiotic prescriptions are associated with the development of antibiotic resistance [3]. It is also important to note that the approval of new antibacterial agents with new mechanisms of action by the Food and Drug Administration (FDA) has declined since 1983 [2]. Hence, it is

necessary to initiate preventive actions against MDR bacteria and to develop new molecules effective against those pathogens in order to reduce the deathrate of people.

Nanoparticles (NPs) that have been shown to effectively target and destroy microbes represent a promising solution [4]. Indeed, metal-based NPs, such as metal-oxide nanoparticles (MONPs), and biosynthesized NPs and their recombinants have been used to overcome limitations of classic antibacterial drugs [5]. Furthermore, studies on metal-based NPs, including those with gold (Au), silver (Ag), iron (Fe), copper (Cu), magnesium (Mg), and MONPs with zinc oxide (ZnO), iron oxide (e.g., Fe_3O_4 or α-Fe_2O_3), titanium dioxide (TiO_2), and cerium oxide (CeO_2), indicate their potential use as novel antimicrobial agents for the control of microorganisms [6]. Metal-based NPs have shown an ability to inhibit bacterial growth and bacterial biofilm formation [7][8][9][10][11][12] and consequently have been proposed as tools to combat infectious diseases [13].

Thus, many studies have abundantly demonstrated the antimicrobial activity of metal-based NPs [4][6][14]. The antimicrobial mechanism of action of metal-based NPs is described as being mainly linked to the following sequence of reactions: attraction to the bacterial surface, destabilization of the bacterial cell wall and membrane, resulting in a change in its permeability, induction of toxicity and oxidative stress by generation of reactive oxygen species (ROS) and free radicals, and, finally, the modulation of signal transduction pathways[15]. Numerous works also show that NP-based materials have been used as a new defense strategy against antimicrobial resistance.

NPs have been described to involve different mechanisms for combating microbial resistance, such as oxidative stress [5]. Major involvement of ROS in antimicrobial therapy could be an interesting antibacterial strategy; therefore, it is essential to fully demonstrate that the metal-based NPs could kill the bacteria via a mechanism of action dependent on the oxidative stress.

2. Mechanistic Insights into the Antimicrobial Actions of Metal-Based Nanoparticles and Oxidative Stress Due to ROS Generation

2.1. Antibacterial Mechanisms of NPs

Most antimicrobial drugs target bacteria by inhibition of (i) cell wall synthesis, with peptidoglycan as the main target [16][17][18], (ii) nucleic acid synthesis, (iii) protein synthesis [19], and (iv) modification of membrane permeability [20]. Bacteria are particularly adept at developing or acquiring resistance mechanisms independent of target functionalities. The various resistance mechanisms included: expression of enzymes capable of altering or degrading antimicrobial agents [21][22], causing cell wall modifications, ribosomal mutations [23], and changes in porin expression (with or without an active efflux mechanism) [24][25].

The use of metal-based NPs as antibacterial agents opened opportunities to develop new strategies against antimicrobial resistance [14]. Even if their antibacterial mechanisms of action are not well elucidated, the majority of the evidence shows that the NPs act when they are in contact with the bacterial cell walls via various antimicrobial

means [26]. It has been described that the negatively charged molecules composing both Gram-positive and Gram-negative cell walls promote the interaction between NPs and the bacterial membrane. These negatively charged molecules have a strong affinity for the positive ions released by most NPs, including AgNPs [27], AuNPs [28], CuNPs [6][29], ZnONPs [30][31], α-Fe_2O_3 metal-based NPs [32], and TiO_2 [33], leading to the electrostatic attraction of NPs to the bacterial surface that induces a disruption of the bacterial cell wall and an increase in its permeability[34]. It was determined that positively charged AgNPs are strongly attracted to the surface of the bacteria, which increases antibacterial activity [27]. Conversely, neutral or negatively charged AgNPs have significantly decreased antibacterial activity [27]. Once the contact between the NPs and the bacterial wall is established, the NPs can then directly cross the bacterial cell membranes, interfere with metabolic pathways, and induce changes in membrane shape and function. Once inside cells, NPs could inhibit enzymes, deactivate proteins, and induce oxidative stress and electrolyte imbalance [35]. NPs can also release metal ions in the extracellular space; these are capable of entering the cell and disrupting biological processes [34]. For example, it has been shown that during the treatment of *Escherichia coli* K12 with AgNPs, NPs interacted with the bacterial cell wall, then dissolved to release Ag^+ into the cell, and finally triggered a transcriptional response that caused more toxicity to the cells [36][37]. It was demonstrated that bacterial DNA was condensed when both *E. coli* and *S. aureus* were exposed to Ag^+, arresting cell multiplication [38]. In addition, there is evidence that exposing bacteria to NPs causes nuclear fragmentation [39] or physical attachment of the AgNPs to the DNA because of the high affinity of Ag^+ to phosphates, which are highly abundant in DNA molecules [40]. Another study reported that *E. coli* treated with AgNPs upregulated many genes covering a wide range of cellular functions, including membrane structure, biofilm formation, the citric acid cycle, electron transfer, cellular transport, and protein efflux [41].

2.2. ROS-Dependent Oxidative Stress

“Oxidative stress” is described as an imbalance between the production and consumption of reactive oxygen species. ROS are chemically reactive species that include a variety of molecules and free radicals derived from molecular oxygen: hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), reactive superoxide radical anion ($O_2{\cdot}^-$), and hydroxyl radicals (·OH) [42][43]. The reduction of molecular oxygen (O_2) produces superoxide anion ($O_2{\cdot}^-$), which is the precursor of most ROS and mediates chains of oxidation reactions. The dismutation of $O_2{\cdot}^-$ leads to the formation of hydrogen peroxide (H_2O_2), which can be totally reduced to H_2O or partially reduced to hydroxyl radicals (·OH). The formation of ·OH is also catalyzed by reducing transition metals (i.e., Fenton reaction). These transition metals can be reduced by the superoxide anion ($O_2{\cdot}^-$), which inevitably leads to the propagation of the cascade of oxidation reactions [44]. Alternatively, superoxide anion ($O_2{\cdot}^-$) can react with nitric oxide radical (·NO) to form reactive nitrogen species, such as peroxynitrite anion ($ONOO^-$), nitrogen oxide radical ($\cdot NO_2^-$), nitrate anion (NO_3^-), and carbonate anion ($CO_3{\cdot}^-$) [45]. This oxidative process can kill microorganisms if hydroxyl radical accumulation is not controlled and leads to oxidative damage of proteins, lipids, and nucleic acids [46]. Bacteria have a number of defense proteins against ROS, involving enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) [47], the thioredoxin system [48], and peroxiredoxin (Prxs) [49]. When this system becomes ineffective for the detoxification of ROS, the bacterial cell oxidizes due to the stressful situation.

2.3. Metal-Based Nanoparticles Target ROS Production in Bacteria

The generation of ROS has been described as an important mechanism of nanotoxicity, resulting in the subsequent induction of oxidative stress in cells and microbial death [50][51]. The level of ROS generation has been evaluated during treatment with nanomaterials, and it has been found to depend on the chemical nature of the NPs [51]. Engineered nanomaterial studies affirm that smaller size and high specific charge of the surface area leads to the production of higher levels of ROS [52][53]. Either metal ions or NPs can induce the production of ROS in the intracellular space, as described by Mazur et al. [54]. Another study on metal-based NPs showed that different NPs, while exhibiting similar inhibitory effects on bacterial growth, can differ significantly in terms of ROS-generated damage [55].

An increasing number of studies show that NP-induced oxidative stress can be exploited for killing a wide range of pathogens; thus, NPs could meet the need for new antibacterials with new mechanisms of action [56]. Several researchers report that AgNPs promote the induction of ROS [50][57]. The relevant antimicrobial effect of AgNPs is attributed to its potential upregulation of ROS, leading to damage of the cell cytoskeleton, proteins, and nucleic acids that eventually results in the antibacterial effect [50][58]. CuNPs are also well known to be highly cytotoxic by the release of ions and the production of ROS [59]. Additionally, other MONPs, such as ZnO [60], TiO_2 [61], and MnO [62], have been proven to exhibit bactericidal activity via their ability to stimulate the cell to produce ROS. In addition to the disruption of bacterial membranes, NPs have been demonstrated to disrupt biofilm formation. In fact, biofilms play an important role in the development of bacterial resistance against antibiotics and other antibacterial agents [63]. Numerous studies have shown that metal-based NPs, including AuNPs [64], AgNPs [65], MgONPs [66], ZnONPs [67], and CuONPs [68], can prevent or overcome biofilm formation.

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