ABSTRACT

In the first part of the study, immunological and antibacterial mechanisms of zinc oxide nanoparticles (ZnO-NPs) were evaluated against human pathogens. ZnO-NPs showed more activity against *Staphylococcus aureus* and least against *Mycobacterium bovis*-BCG. However, BCG killing was significantly increased in synergy with antituberculous-drug rifampicin. Antibacterial mechanistic studies showed that ZnO-NPs disrupt bacterial cell membrane integrity, reduce cell surface hydrophobicity and down-regulate the transcription of oxidative stress-resistance genes in bacteria. ZnO-NP treatment also augmented the intracellular bacterial killing by inducing reactive oxygen species production and co-localization with *Mycobacterium smegmatis*-GFP in macrophages. Moreover, ZnO-NPs disrupted biofilm formation and inhibited hemolysis by hemolysin toxin producing *S. aureus*. Intradermal administration of ZnO-NPs significantly reduced the skin infection, bacterial load and inflammation in mice, and also improved infected skin architecture. We envision that this study offers novel insights into antimicrobial actions of ZnO-NPs and also demonstrates ZnO-NPs as a novel class of topical anti-infective agent for the treatment of skin infections.

In order to improve the chemotherapy of tuberculosis, there is an urgent need to enhance the efficacy of existing agents and also to develop more efficient drug delivery systems. In the second part a novel anti-TB drug complex was synthesized which consists of zinc and rifampicin (Zn-RIF). The synthesized drug complex was encapsulated into transferrin-conjugated silver quantum-dots (Zn-RIF-Tf-QD) to improve delivery in macrophages. Successful synthesis of Zn-RIF and Zn-RIF-Tf-QD was confirmed by UV/Vis-spectroscopy, TEM, FTIR, photoluminescence, XRD, XPS, and NMR. The sizes of silver QDs and transferrin-conjugated QDs were found to be in the range of 5-20 nm. Activity assays showed that Zn-RIF-Tf-QD exhibited 10-fold higher antibacterial activity against *Mycobacterium smegmatis* and *Mycobacterium bovis*-BCG as compared to Zn-RIF, RIF and Zn. Immunofluorescence studies showed that Zn-RIF-Tf-QD-conjugates were actively endocytosed by macrophages and dendritic cells, but not by lung epithelial cells. Treatment with Zn-RIF-Tf-QD efficiently killed mycobacteria residing inside macrophages without exhibiting cytotoxicity and genotoxicity. Moreover, the conjugates remained stable for upto 48 h, were taken up into the late endosomal compartment of macrophages, and released the drug in a sustainable manner. The results demonstrate that Zn-RIF-Tf-QDs have a great
potential as anti-TB drugs. In addition, transferrin-conjugated QDs may constitute an effective drug delivery system for tuberculosis therapy.

Zinc oxide nanoparticles (ZnO-NPs) have wide biological applications, which have raised serious concerns about their impact on the health and environment. Although, various studies have shown ZnO-NP toxicity on different cells under in-vitro conditions, sufficient information is lacking regarding toxicity and underlying mechanisms under in-vivo conditions. In the third part, genotoxic, clastogenic, and cytotoxic effects of ZnO-NPs were evaluated on macrophages and in adult mice. ZnO-NP treated mice showed signs of toxicity such as loss in body weight, passive behavior and reduced survival. Further mechanistic studies revealed that administration of higher dose caused severe DNA damage in peripheral blood and bone marrow cells as evident by the formation of COMET tail, micronuclei, chromosomal fragmentation, and phosphorylation of H2A histone family member X. Moreover, ZnO-NPs inhibited DNA repair mechanism by downregulating the expression of fen-1 and pol β proteins. Histopathological examinations showed severe inflammation and damage to liver, lungs, and kidneys. Cell viability and wound healing assays revealed that ZnO-NPs killed macrophages in a dose-dependent manner, caused severe wounds and inhibited cellular migration by irreversible actin depolymerization and degradation. Reduction in the viability of macrophages was due to the arrest of the cell cycle at the G0/G1 phase, inhibition of superoxide dismutase and catalase and eventually reactive oxygen species. Furthermore, treatment with an antioxidant drug N-acetyl cysteine significantly reduced the ZnO-NP induced genotoxicity both in-vitro and in-vivo.