Immunome Research
ISSN: 1745-7580

Raguvaran et al., Immunome Res 2015, 11:2
DOI: 10.4172/1745-7580.1000095

Research Article Open Access

Zinc Oxide Nanoparticles: Opportunities and Challenges in Veterinary Sciences

Raguvaran R, Anju Manuja* and Balvinder Kumar Manuja

ICAR-National Research Centre on Equines, Sirsa road, Hisar, Haryana, India

***Corresponding author:** Anju Manuja, ICAR-National Research Centre on Equines, Hisar-125001, Haryana, India, Tel: +911662276748; Fax: +91 1662 276217; E-mail: amanuja@rediffmail.com

Received date: March 26, 2015; **Accepted date:** June 27, 2015; **Published date:** June 30, 2015

Abstract

Nanotechnology has opened up new vistas for applications in almost all the disciplines of veterinary and animal sciences. Zinc oxide (ZnO) has been used as an alternative to antibiotics in animal feed. It has also been widely used for wound healing and various skin disorders. Recently ZnO nanoparticles (NPs) have attracted attention owing to their unique features. There can be numerous applications of ZnO NPs due to their antibacterial, antineoplastic, wound healing, ultraviolet scattering and angiogenic properties. These have also been used to promote tissue repair, as a food preservative and as feed additive. This paper reviews the recent developments in ZnO NPs research and its potential for application in animal health and production.

Keywords: Nanotechnology; ZnO nanoparticles; Antitumor activity

Introduction

Nanotechnology has become the forefront of research and has the tremendous potential to revolutionize the livestock sector. With the advent of this emerging field, wide varieties of nanoparticles with exciting characteristics are being manufactured and used for a broad range of applications [1]. The amount of manufactured nanoparticles will rise sharply to 58,000 tons by 2020 [2]. The metal oxide nanoparticles are most commonly used in catalysis, sensors, environmental remediation and personal care products [3,4]. Zinc oxide (ZnO) among nanosized metal oxides has been extensively used due to its antimicrobial and antitumor activities [5]. ZnO is generally included in cosmetic lotions as it is known to possess UV absorbing and blocking properties [6]. ZnO has also been used as an astringent for eczema, excoriation, wounds and haemorrhoids in human medicine [7]. Recently ZnO NPs have attracted attention owing to their unique features. There can be numerous potential applications of ZnO NPs in veterinary sciences due to their antibacterial, antineoplastic, wound healing and angiogenic properties. ZnO NPs have been used in tissue repair, as food preservative and as feed additive (Figure 1). In animals, the treatment of meningitis, tumors and diseases caused by intracellular pathogens like viruses, bacteria (*Brucella*, *Chlamydia*, and *Rickettsia* etc.) and fungi (*Histoplasma capsulatum*, *Cryptococcus neoformans*) is really difficult due to inaccessibility of the drugs to the target site. To treat these affections the therapeutic agents have to penetrate inside cells and able to cross the blood brain barrier. This is not possible with existing macro molecular therapeutic substances, but the nanoparticles can act against intracellular pathogens and brain tumors due to their small size [8]. This review deals with the potential applications of ZnO NPs in veterinary sciences. Numerous conditions in animals where ZnO NPs may play role and the potential uses of ZnO NPs as food preservative and feed additive have also been described. Finally, the challenges in terms of the potential toxic effects of ZnO NPs have also been discussed.

Figure 1: Schematic presentation of potential applications of Zinc oxide nanoparticles (ZnO NPs) in veterinary sciences due to their antibacterial, antineoplastic, wound healing and angiogenic properties. ZnO NPs have also been used in tissue repair, as food preservative and feed additive.

Antibacterial Property

Commonly used antibiotics have narrow therapeutic index and their continuous usage leads to antimicrobial resistance [9]. The novel substitutes especially inorganic nanoparticles have recently attracted attention. Metal oxide in the nano-range acquires distinctive properties that depend on size, chemical composition and surface chemistry. Among the metal oxide nanoparticles, the ZnO NPs have been commonly used for their antimicrobial properties. ZnO has been found to reduce the activity of wide range of bacteria [10,11]. With the use of NPs the antibacterial property increased significantly [12-14]. The precise mechanism of antibacterial action is not yet understood, but four mechanisms have been hypothesized as illustrated in Figure 2. Firstly, affinity between ZnO and bacterial cells [15]; Secondly, microbial cell injury caused by hydrogen peroxide generated from the surface of ZnO [12,16,17]. Thirdly, upon penetration into bacteria ZnO NPs cause bactericidal activity by interacting with phosphorus and sulphur containing compounds like DNA of bacteria [18-21]. Last but not the least, ZnO NPs arrest the cellular metabolism of bacteria by binding to the protein molecules and ultimately causing death of the microbes [22].

ZnO NPs have more pronounced antibacterial property due to small size and high surface-to-volume ratio. The size of the nanoparticles may have greater impact on their activity due to more accumulation inside the cell membrane and the cytoplasm [23]. Comparative study on antibacterial effect of both micron and nano scale particles of ZnO revealed that ZnO NPs have a greater antibacterial effect [24]. ZnO NPs could be ideal for replacing some of the existing antibiotics [25-28].

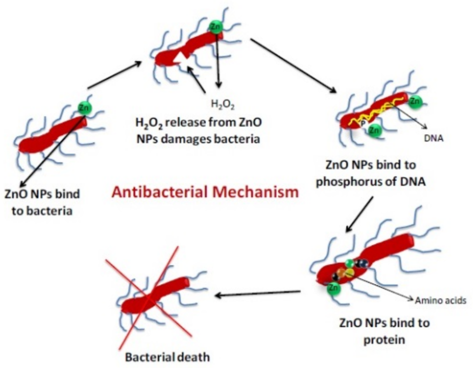

Figure 2: Diagrammatic presentation of possible mechanisms of antibacterial action of Zinc oxide nanoparticles (ZnO NPs). ZnO NPs bind to the bacterial cells. The hydrogen peroxide generated from the surface of ZnO cause microbial cell injury and nanoparticles penetrate into bacteria. ZnO NPs cause bactericidal activity by interacting with phosphorus and sulphur containing compounds like DNA of bacteria. ZnO NPs arrest the cellular metabolism of bacteria by binding to the protein molecules and ultimately causing death of the microbes.

ZnO NPs can have application in conditions like mastitis in animals, where they can reach the target organisms generally inaccessible to conventional antibiotics. Mastitis is a disease of high yielding animals commonly caused by *Staphylococcus*, *Streptococcus* and *E. coli*. Economic consequences of mastitis include reduced milk yield and increased use of drugs and veterinary services [29,30]. It is really very difficult to treat mastitis due to antibiotic resistance. Presence of biofilm inside the udder tissue makes the bacteria impervious to physical, chemical and even innate immune mechanism [31]. ZnO NPs have been found to be effective against *S. aureus* and *E. coli* [32-34]. The treatment of staphylococcal mastitis in animals is really challenging due to intra cellular tendency of the organism. As the nanoformulations are able to penetrate the cell, ZnO NPs can be employed for staphylococcal mastitis. Most of the times, the pH of mastitis milk is alkaline in nature. The antibacterial with higher concentration in alkaline medium will be right choice to tackle this situation. Antibacterial activity of ZnO NPs against *S. aureus* was found to be greater at basic pH [32].

E. coli is a gram negative bacterium responsible for coliform mastitis in ruminants, white scours in calves and coli granuloma in birds. ZnO NPs have strong antibacterial activity against *E. coli* [35-37]. Fowl typhoid is a vertically transmitted disease caused by *Salmonella gallinarum* in chicken all over the world [38]. ZnO NPs were found to be effective against *Salmonella typhimurium* and *Staphylococcus aureus* [34,39]. Listeriosis, also known as circling disease is caused by *Listeria monocytogenes* characterised by encephalitis, septicaemia and abortion in ruminants. ZnO NPs were effective against *L. monocytogenes* at various concentrations [40].

Pseudomonas aeruginosa is a gram negative, rod shaped organism and prefers to reside in moist anatomic sites such as respiratory and gastrointestinal tracts in animals and human being [41,42]. It is intrinsically resistant to wide range of antimicrobials like β-lactam, tetracycline and chloramphenicol due to drug efflux mechanism and presence of porin [43,44]. Biofilm formed by this organism makes another obstacle to antimicrobials [45]. ZnO NPs were found to be effective against *Pseudomonas* [32].

Bacillus anthracis, a spore forming bacteria, causes anthrax in warm-blooded animals. On the other hand, *Clostridium* an anaerobic spore forming microbe is responsible for various diseases like tetanus, botulism, enterotoxaemia, black quarter and braxy in animals. The spores produced by these organisms remain viable in the environment for prolonged period and mature to vegetative bacilli under favourable condition. These spores are highly resistant to extreme temperature and pressure. ZnO NPs have been found to be effective against the bacterial spores [46].

ZnO NPs synthesized from plant source revealed enhanced antimicrobial activity against various pathogens as compared to chemical ZnO NPs. The activity increased with increased dose and treatment time [47].

Antineoplastic Property

Amongst the existing treatment strategies for cancer, the chemotherapeutic agents are the most commonly used in clinical patients. Currently existing antineoplastics are not highly effective in curing the patients due to lack of selective toxicity. Indiscriminate use of antineoplastics may leads to devastating adverse effects like bone marrow suppression, neurotoxicity and cardiomyopathy [48,49].

So, there is great emphasis to develop novel anti-cancer agents that can selectively target cancer cells while sparing normal cells. Nanotechnology has opened new vistas for the treatment and diagnosis of cancer [50-52]. Nanoparticles can be employed as vehicles for targeted delivery to tumour sites [53]. The prime benefit is that they can be taken up by specific cells and internalized to the nucleus according to their surface chemistry.

At appropriate concentration ZnO NPs induced the production of variety of proinflammatory cytokines like TNF-α, IFN-γ and IL-12 in *in vitro* and *in vivo* (rat) pulmonary inhalation studies [54-57]. Cytokines induced by nanoparticles could also facilitate effective anti-cancer actions by eliciting a cytokine profile appropriate for directing the development of Th1-mediated immunity [58].

ZnO NPs have been shown to possess cell selective toxicity having preferential killing of cancer cells with minimal toxicity to normal primary immune cells [2,53,59]. ZnO NPs based diagnostic devices are useful in the detection of low level of biomarkers for cancer diagnosis [60,61]. These applications of ZnO NPs can easily be extrapolated for diagnostic and therapeutic purposes in common neoplastic conditions of animals like lymphoma, cutaneous cancer, transmissible veneral tumor and equine sarcoids.

Neoplasm of integumentary system is the most commonly encountered neoplastic disorder in domestic animals [62,63]. The harmony between cutaneous cancer and ultraviolet radiation has been well established in domestic animals [64-66]. In canines, deeply penetrating ionizing radiations induce blood and lung cancer [67]. Among the haematopoietic tumours, canine lymphoma is the most common in dogs [68,69] and manifested as generalized lymphadenopathy [70]. Canine transmissible veneral tumor also called as sticker tumor is spread through abraded skin or mucosa during coitus and also by licking [71]. Apart from facial region, it can also occur in skin and anal mucosa [72-75]. Metastasis is rare with this tumor but, involvement of regional lymph nodes and vital organs has been reported [76-82]. Tumour of mammary gland is often noticed in intact bitch [83]. In equines, equine sarcoid is the most common fibroblastic skin tumour affecting horses, mules and donkeys [84-86]. The infection is highly correlated with bovine papilloma virus type 1 and 2 [87,88]. The use of ZnO NPs may be explored for diagnostic as well as therapeutic approaches in these conditions.

Wound Healing

A wound by true definition is a breakdown in the protective function of the skin; the loss of continuity of epithelium, along with or without loss of underlying connective tissues including muscle, bone and nerves [89]. The sequences of wound healing comprise inflammation, epithelization, granulation and tissue remodelling irrespective of the etiology [90]. Microbial contamination of wound leads to delayed healing process [90-93].

Dog bite wounds are very often contaminated by diversity of microorganisms from different sources [94,95]. In chronic wounds, bacteria persist in biofilm form and they induce chronic inflammation which delays healing and make them more resistant to antimicrobial therapy [96]. In case of burn injury, affected animals are highly prone to bacterial infection and ultimately lead to severe septicaemia and death. It is reported that around 75% of mortality following burn injuries is associated with *Pseudomonas aeruginosa* or methicillin resistant *Staphylococcus aureus*. Interestingly, ZnO NPs were found to be effective against *Pseudomonas* and *S. aureus* and can be used for injuries infected with these organisms [32].

Topical application of antiseptic preparations is the best for wound treatment because of their direct action at wound site [97-99]. Topical application of zinc has been reported to improve re-epithelialization, reduce inflammation and bacterial growth in case of leg ulcers [100]. ZnO possesses both antibacterial and anti-inflammatory properties and accelerates the healing of both acute and chronic wounds [36,101]. It has potential application in the treatment of various conditions like dermatitis, blisters and open skin sores [102-104]. ZnO NPs are superior to conventional ZnO powder in terms of antimicrobial activities especially against Gram-positive organisms [34].

Nervous Tissue Repair

Nerve guidance channel (NGC) is a type of implant placed around the damaged nervous tissue to promote the healing. It is a hollow tube into which two severed ends of nerve fibre bundles are inserted and sutured into place. NGC provides congenial milieu that promotes or accelerates tissue healing. Conductive and piezoelectric materials such as polyvinylidene fluoride have been used to enhance the neurite outgrowth and regeneration of axon without any external electrical stimulation [105-107]. Incorporation of nanoscale [108] and piezoelectric elements [105] into the NGC independently enhance the neural regeneration.

Regeneration of CNS after injury is inhibited mainly due to astrocytic glial scar formation. Implant placed outside of the CNS such as an orthopedic implant may attract fibroblast cells and compromise the osseointegration. Astrocyte activity was minimized by composites made of ZnO nanoparticles and polyurethane [109]. These properties of ZnO NPs can be exploited for diseases of CNS in animals.

Food Preservation

Microorganisms such as *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* sp are the most common food borne pathogens and continue to draw public attention. Each year around 22.8 million cases of salmonellosis and 37,600 mortalities have been estimated in South East Asia [39]. In the present scenario, the ready to eat food products are getting popular because of convenience. They are highly prone to moisture loss, browning and biofilm action by microbes [110]. Contamination with microbes like *E. coli*, *Salmonella* and *Staphylococcus aureus* has been reported regularly in food products [39,111]. To mitigate the growth potential of foodborne pathogens, implementation of food safety management system is essential [112]. As the incidence of food borne infections is increasing all over the world, the safety of livestock products is important. Preservatives play an important role in storage and transport of perishable livestock products. Antimicrobial compounds in food packaging system recently attracted attention due to consumer safety and prolonged shelf life of the product [111]. Currently the food industries are utilizing organic and inorganic substances for food preservation. The inorganic antibacterial agents can withstand high temperature and pressure better than organic substances [17]. High stability and safety makes ZnO a candidate for use in food preservation [15,25].

Apart from silver nanoparticles zinc, copper and titanium are also used in packaging industry. ZnO NPs have been used as an antimicrobial agent in food packaging [33]. Antibacterial activity of ZnO NPs coated polyvinyl chloride has been observed against *E. coli* and *S. aureus* [113].

Feed Additive

Feed additive can be defined as a substance not having a direct utilisation as a nutrient, but included at an optimum concentration in diet or in the drinking water to exert positive action on the animal health or on the dietary nutrient utilisation. As an alternative to existing feed additives antibiotics are used due to their low cost and uniform response. Use of antibiotics in diet of pigs increased the weight gain and reduced feed conversion ratio by 0.16 and 0.07 respectively [114]. But continuous use of antibiotics provokes retention in animal tissues and subsequent consumption of such animal product will certainly increase risk of antibiotic resistance in humans.

Post-weaning diarrhoea (PWD) caused by enterotoxigenic *E. coli* (ETEC) is a major health concern in weaner piglets. It is associated with an increase in morbidity and mortality and decrease in growth rate during the weaning period [115]. The addition of zinc (zinc oxide) at the concentration of 2500 to 3500 ppm in feed modulated the microbial status of the digestive tract and reduced the incidence of post-weaning diarrhoea in piglets [116,117] and increased productive performances [118,119].

ZnO has been found to be effective against PWD at a concentration of 3 kg/ton of feed, but the major limitation was high concentrations of zinc in faeces [119,120]. Supplementation of 100 ppm microencapsulated ZnO was highly effective when compared to supplementation of 3,000 ppm ZnO for suppression of PWD in the first 2 weeks after weaning and also reduced plasma zinc concentration and faecal zinc excretion levels [121]. It is obvious that the same effect may be achieved with lesser quantities of ZnO nanoformulation in the feed.

Ultraviolet Scattering

Ultra violet rays UVA-1 (340-400 nm) and UVA-2 (320-340 nm) upon reaching our skin cause several biological and metabolic reactions [122,123]. The dermal part of the skin plays a vital role in the photo aging process. Reactive oxygen species (ROS) produced by UV rays activate different matrix metalloproteinases that subsequently damage collagen and other dermal matrix proteins [124,125]. Long-term exposure leads to degenerative skin changes, actinic keratoses and skin cancer. UV-B causes cancer in animals similar to those observed in humans. Heavy coats and skin pigmentation of most animals provide protection from UV-B, but eyes and exposed parts of the body are considered at risk.

Inorganic materials have proven efficacy against UV induced skin damage. Benefits offered by sunscreens based on inorganic compounds include absence of skin irritation and sensitization, limited skin penetration and a broad spectrum protection. Sun screen lotion containing nanoparticles of Titanium dioxide (TiO2) and ZnO are transparent and this property provides the cosmetic acceptability not achievable with opaque formulations [126].

Angiogenesis

Ischaemia is defined as local deficiency of arterial blood supply to an organ. It is caused by several factors like alteration in the wall, external pressure from abscess, cyst, and haematoma and blockage of lumen. It is also noticed in valvular thrombi of heart in pigs caused by *Erysipelothrix rhusiopathiae*, mural thrombi of heart in cattle caused by *Clostridium chauvoei*, verminous aneurysm of anterior mesenteric artery in horse caused by *Strongylus vulgaris*, *Spirocerca lupi* infection of aorta in dog, *Onchocerca armillata* affection in the aorta of cattle and finally in the ergot poisoning. Persistent ischaemia leads to necrosis or atrophy of affected organ. So, it is important to restore the perfusion of affected organ.

Angiogenesis is the process of formation of new capillaries from pre-existing blood vessels, involving both pro- and anti-angiogenic factors. It plays an important role in embryonic development, wound repair, post-ischemic vascularisation of the myocardium, tumor growth and metastasis [127-129]. Vascular endothelial growth factor A (VEGF-A) and basic fibrobroblast growth factor (bFGF) are the pro angiogenic factors used for the treatment of cardiovascular disease, ischemia and wound healing [127,130-133]. But their limitation with usage is associated with pathological angiogenesis, thrombosis, fibrosis and/or the tumor cells proliferation [134]. So, there is a great emphasis to develop novel pro-angiogenic factors which will specifically promote angiogenesis in required area without affecting normal tissue. ZnO nanoflowers have exhibited significant angiogenic property both *in vitro* and *in vivo* in chick embryo [135].

Safety Concerns with ZnO NPs

The properties that offer promise for the development of new technologies also pose an unknown risk to animals and environmental health. FDA considered ZnO as a generally recognized as safe substance (GRAS). The GRAS designation mostly encompasses materials ranging from micron to larger size. So, conversion of these macro substances to nanoscale will attain distinctive properties including toxicity. ZnO NPs tend to dissociate and release ions when exposed to a biological environment resulting in reactive oxygen species (ROS) production and cellular oxidative stress [136,137]. Zinc is an essential component of enzymes (e.g. carbonic anhydrase, alcohol dehydrogenase, matrix metalloproteinase) and transcription factor like zinc finger protein transcription factors. Alteration in cellular Zn homeostasis in *in vitro* systems leads to loss of viability, oxidative stress and mitochondrial dysfunction [138]. ZnO NPs rapidly dissolve in acidic milieu and produce a high local concentration of Zn^{2+} ions.

Zn^{2+} is an essential element in cell homeostasis and remains in the bound form inside cells because free Zn^{2+} is very reactive and cytotoxic [139]. The sudden elevation of free Zn^{2+} levels may damage lysosomes allowing the contents to release into the cytoplasm and ultimately causing cell death [140,141]. ZnO NPs exhibit protein adsorption property which might be responsible for their cytotoxicity [142]. The pH-triggered (acidic) intracellular release of Zn^{2+} is mainly responsible for the toxicity of ZnO nanowires [143].

The pathological changes induced by ZnO NPs mainly depend on size and dose [144]. ZnO NPs were found to be toxic to all human or rodent cells when the concentration was above 15 ppm [145]. ZnO NPs have been studied extensively and they affect different cell types and animal systems [146-151]. They are toxic to animals after intra tracheal instillation [152] inhalation [153] and after oral administration [154,150]. Comparative study on kinetic properties of ZnO NPs with simple ZnO powder revealed that single intraperitoneal administration of 100 nm sized ZnO NPs (2.5 g/kg b.wt) accumulated in liver, spleen, lung, kidney, and heart and the concentration was higher than the administration of similar amounts of 1 μm sized ZnO particles [155].

We have demonstrated that interaction of different concentrations of ZnO NPs with horse erythrocytes revealed the absence of hemolysis by spectrophotometric method. While, phase contrast microscopic examination revealed concentration dependent clustering of erythrocytes [156]. It warrants the detailed investigation of interaction of erythrocytes with ZnO NPs before their applications in animals. There are still many challenges to be faced in terms of the potential toxic effects. Incorporation of metal nanoparticles into polymeric hydrogel matrices reduce the toxicity and improve the efficacy because of sustained and controlled release. The effective delivery of ZnO NPs can be achieved by entrapping, attaching or encapsulating into the nanoparticle matrix to prevent undesirable effects. We have observed that nanoformulation of ZnO NPs using biocompatible polymers enhances the efficacy at lower doses with sustained release [157]. The toxicity of ZnO NPs-loaded nanohydrogels was substantially lower compared to ZnO NPs.

Conclusion

We described various animal diseases, where antibacterial, antineoplastic, and angiogenic properties of ZnO NPs can be exploited. These have also been used to promote tissue repair, as a food preservative and as feed additive. Conversion of these macro substances to nanoscale will attain distinctive properties including toxicity. A discussion regarding the advantages, approaches and limitations on the use of ZnO NPs for various applications and drug delivery has been presented. The understanding of challenges in terms of the potential toxic effects of ZnO NPs, the possible mechanisms and cellular consequences as a result of ZnO NPs interactions with host cells is necessary to provide better delivery options for ZnO NPs. The approaches discussed to improve their safety will further make them attractive for various applications in veterinary species.

Acknowledgement

The authors thank Indian Council of Agricultural Research, Ministry of Agriculture, Government of India, New Delhi for providing financial support. Raguvaran is thankful to ICAR for providing the fellowship during his MVSc.

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