Investigation of Zinc Oxide Nanoparticles as Anti-cancer Drug Delivery System

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Abstract
ZnO nanoparticles have an advantage over soluble polymers as drug carriers because they can remain in the body for considerable periods of time. Many studies have demonstrated that Zinc Oxide (ZnO) nanoparticles hold promise as anti-cancer agents. The mechanism for cytotoxicity caused by ZnO is not completely understood. The toxic effect of ZnO nanoparticles with a mean diameter of 20 nm was investigated for the heart tissue of male Wistar rats. Microscopic examination revealed that the nanoparticles caused tissue damage and resulted in alterations. The results demonstrated the toxicity of ZnO nanoparticles and showed that nanoparticle dosage is a critical factor that influences their toxic effects.

Keywords: Zinc Oxide, nanoparticles, toxicity, drug delivery.

Introduction

Anti-cancer therapies usually employ anti-metabolites, biological agents, alkylating agents and natural products. However, they often fail to produce an effective response because they cannot differentiate between tumor and healthy cells. Their indiscriminate effect limits the maximal allowable dose that can be used for a chemotherapeutic drug.[1,2] Studies have shown that zinc oxide (ZnO) nanoparticles exhibit a high degree of selectivity, the ability to surpass the therapeutic indices of some commonly used chemotherapeutic drugs.[3,4]

Nanoparticles can increase the circulation half-life of drugs to several hours. Nanoparticle drug carriers also have the added advantage of being small enough to pass through the capillaries yet being large enough not to go through endothelial gap junctions. But, ZnO nanoparticles have a tendency to build up in the body and can cause organ damage or can breakdown in unpredictable ways. The Food and Drug Administration has classified zinc oxide (ZnO) as "generally recognized as safe substance" ("GRAS").

ZnO when reduced to the nanoscale, can develop new ways to cause toxicity. A detailed evaluation of toxicity in both *in vitro* and *in vivo* systems is needed. Several studies have reported that nano-sized ZnO particles display more serious toxic effects than bulk ZnO[5] and have suggested that size and dose are important in influencing toxic effects. The dosage of nanoparticles is directly related to the effect on the cell biomolecules.[6] This study investigated the toxic effect of ZnO nanoparticles on the hearts of male Wistar rats using three different doses of ZnO nanoparticles for 10 days.

Material and Methods

Synthesis and Characterization of ZnO Nanoparticles: The nanoparticles were prepared by the drop wise addition of 0.6 M NaOH solution to 0.2 M $Zn(CH_3COO)_2$ dissolved in 2-propanol at a temperature of 35-37C°.[7] The characterization of ZnO nanoparticles was performed at King Abdullah Institute for Nanotechnology (Riyadh, Saudi Arabia) (Figure 1). X-ray diffraction data was recorded by using Cu Kα radiation (1.54 A°). The intensities of the diffraction spots were collected over a range of 20-80° and the average particle size of the ZnO nanoparticles was estimated by the Scherrer equation.[8]

The shape of nanoparticles was examined by transmission electron microscopy TEM (Figure 2).

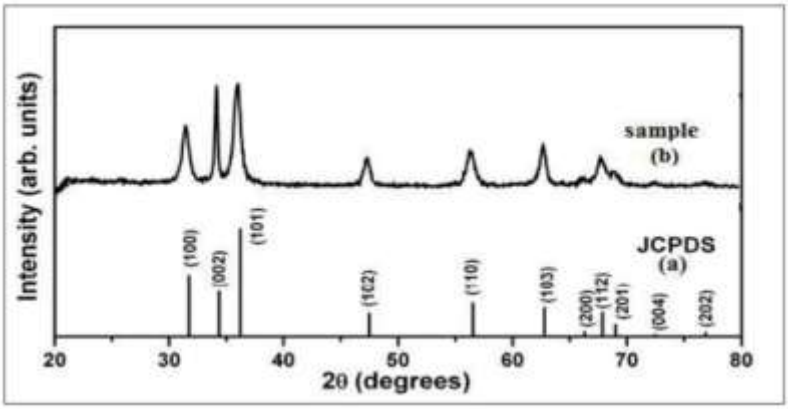

Figure 1: (a) Standard X-ray diffraction pattern of Zinc Oxide according to Joint Commission for Powder Diffraction Standards (JCPDS) and (b) the experimentally acquired X-ray diffraction pattern of ZnO nanoparticles.

Small Animal Studies: The ZnO nanoparticles were added to normal saline buffer to prepare a stock sample with a concentration of 500 mg nanoparticles/mL at a pH of 7.3. The doses of ZnO nanoparticles were then prepared with concentrations comprising 100, 200 and 400 mg/mL in normal saline solution. The experiments were carried out on male Wistar rats (after receiving ethical approval from Vice Dean, Faculty of Applied Medical Sciences, Northern Border University) of about 6 weeks in age and weighing 100-150 g.

The rats were housed in clean and properly ventilated cages under controlled climatic conditions. The rats were divided into four groups. The group 1 (n=6) was control and comprised rats receiving intramuscular injections of saline solution for 10 days. The group 2 (n=6) consisted of rats receiving intramuscular injections of low dose (100 mg ZnO nanoparticles/kg) for 10 days. The group 3 (n=6) received intramuscular injections of medium dose (200 mg ZnO nanoparticles/kg) for 10 days; and the last group 4 (n=6) comprised rats receiving intramuscular injections of high dose (400 mg ZnO nanoparticles/kg) for 10 days.

Histopathological Analysis: On the 11th day, the rats were sacrificed and their hearts were excised. The samples were fixed, washed, dehydrated, cleared and embedded in paraffin wax. Tissue sections with a thickness of 5 μm were prepared for light microscopic examination by applying hematoxylin and eosin stain (H & E).[9]

Results

The X-ray diffraction pattern of ZnO sample compared well with the published diffraction pattern for ZnO reported by the Joint Commission for Powder Diffraction Standards (JCPDS) (Figure 1). The morphology of ZnO nanoparticles was investigated by the use of Transmission Electron Microscopy (TEM). They displayed a hexagonal shape and the measured particle size was 20 nm (Figure 2).

The excised hearts of the treated rats with ZnO nanoparticles showed fragmented muscle fibers. The endomysium appeared to increase in size with an increase in nanoparticle dosage. Also, split muscle fibers and darkened nuclei were visible. When a high dose of ZnO nanoparticles (400 mg nanoparticles/kg) was administered, a loss of normal muscle fiber arrangement followed by areas of myolysis, thinning and shortening of muscle fibers became visible (Figure 3).

Discussion

It is believed that generation of reactive oxygen species (ROS) is involved in the tissue damage caused by ZnO nanoparticles (Figure 4). ROS production can cause oxidative damage to biomolecules and can lead to cell death.[10] A model has been proposed to explain oxidative stress generation by ROS.[11] According to this model: (i) there is an increase in amount of antioxidant enzymes followed by (ii) an increase in cytokines that leads to inflammation and damage to mitochondria of the cells that causes apoptosis or necrosis.[12-15]

Heavy metals are known to have toxic effects which damage the tissue subcellular structures.[16-19] Some studies have reported the toxicity of ZnO nanoparticles in bacteria, macro-algae, yeast, protozoa, zebrafish and mice.[20-23] There have also been reports of damage caused by free Zn^{2+} ions.[24,25] Other reports indicate that Zn^{2+}ions are not the major source of cytotoxicity.[26]

In the human body, Zinc is important for normal growth and developmental processes as well as regulation of the immune system.[27,28] All cells contain a large amount of zinc that is bound as Zn^{2+} to various proteins. Usually, the level of free Zn^{2+} ions is very low and tightly regulated by homeostatic mechanisms.[29,30] An excessive amount of zinc can be harmful and can cause neuronal toxicity and brain injury.[31] When appreciable amounts of ZnO nanoparticles are present, mitochondrial damage and disruption of cellular zinc homeostasis can result that can lead to cell death.

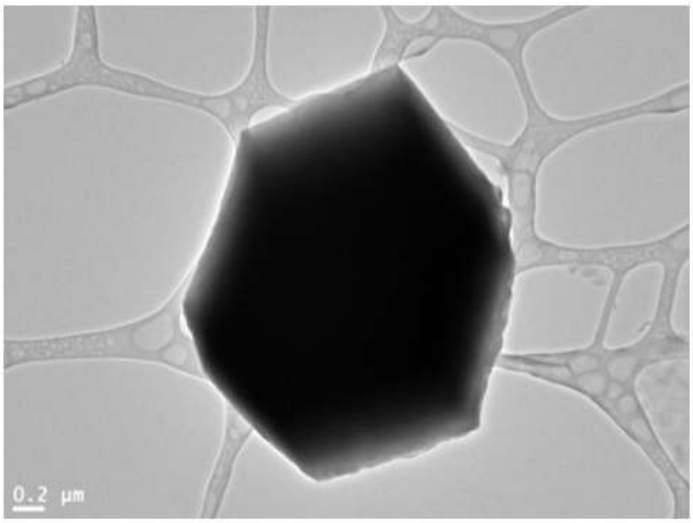

Figure 2: Image of a single ZnO nanoparticle (according to 200 nanometer scale bar) acquired by the use of transmission electron microscope.

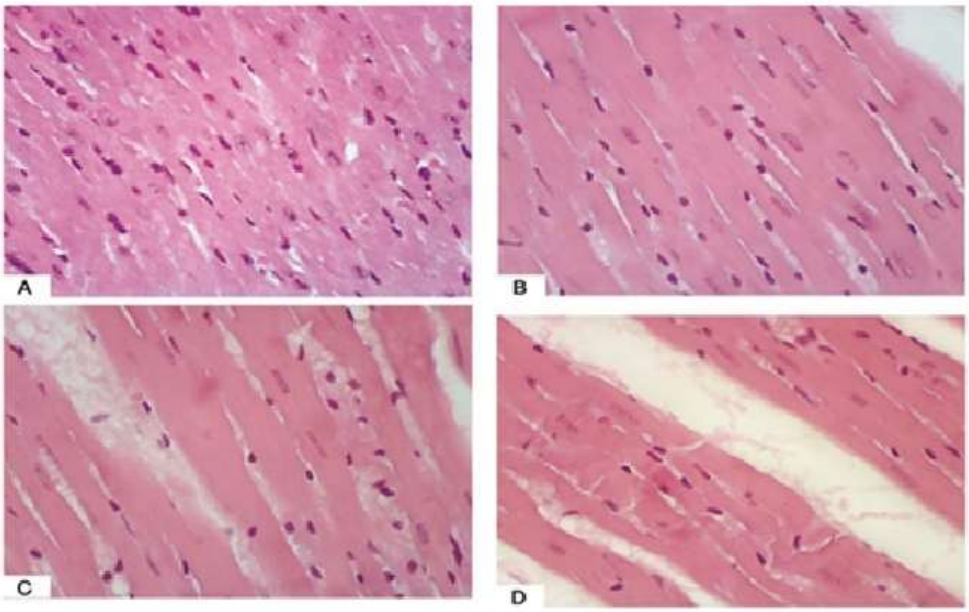

Figure 3: Histopathological sections of heart muscle fibers of male Wistar rats (H & E stain). A: Control group. B: Appearance of male Wistar rat heart muscle fibers after intramuscular injection of 100 mg ZnO nanoparticles/kg daily. C: Appearance of heart muscle fibers after 200 mg ZnO nanoparticles/kg administered daily. D: Appearance of heart muscle fibers 400 mg ZnO nanoparticles/kg administered daily.

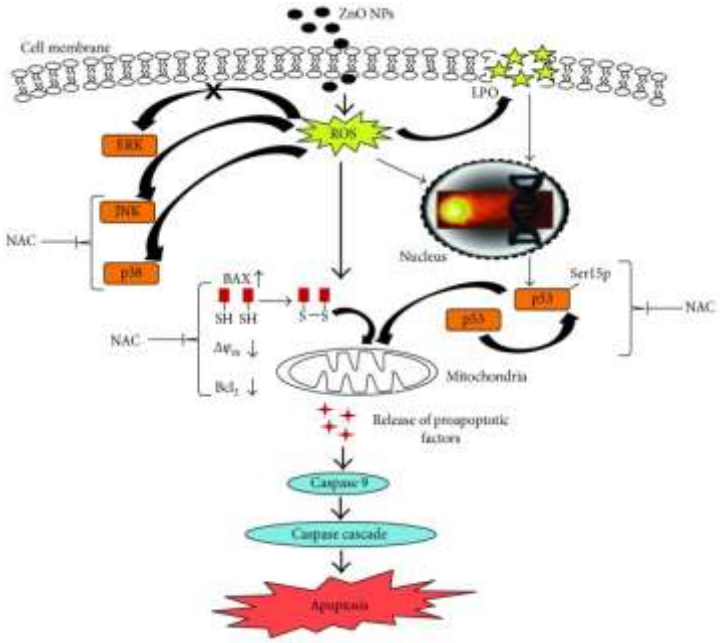

Figure 4: Proposed mechanism by which ZnO nanoparticles kill human cells.

The experimental results of this study indicate that ZnO nanoparticles induce dose-dependent cytotoxicity in the heart tissue and cause histopathological damage (Figure 3). The control group showed normal tissue structure whereas the ZnO-treated group showed histopathological changes.[32] The histopathological changes appeared to be dose-dependent.[33] A decrease in particles size increases the surface area of the nanoparticles, which can cause not only the accumulation of nanoparticles in different tissues, but also increases the reactivity of nanoparticles towards tissue cells.[6]

Conclusion

This study has demonstrated that ZnO nanoparticles with a mean diameter of 20 nm can cause tissue damage in male Wistar rats. A direct proportional relationship between the dosage of ZnO nanoparticles and uptake by the cells was found that led to the accumulation of nanoparticles and resulted in tissue damage.

Acknowledgement

The authors wish to acknowledge the financial support for this study by the research grant no. 435-014-2 awarded by

the Deanship of Scientific Research at Northern Border University, Arar, K.S.A. We also want to acknowledge the help of Dr. Nermin El-Morshedi in the preparation of this manuscript.

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(Received 31st December 2019, accepted 12th March 2020)