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Anticancer studies of synthesized ZnO nanoparticles against human cervical carcinoma cells

Muthuraman Pandurangan, Gansukh Enkhtaivan, Doo Hwan Kim *

Dept of Bioresources and Food Science, Konkuk University, Seoul, South Korea

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ABSTRACT

A metal oxide nanoparticle has been widely investigated for its potential use in the biomedical application. The present study investigates the cytotoxicity of ZnO nanoparticle in human cervical carcinoma cells. Cell viability was determined, and it showed the possible cytotoxic effect of ZnO nanoparticles. The characteristic apoptotic features such as rounding and loss of adherence were observed in the treated cells. Fluorescence and Confocal Laser Scanning Microscope (CLSM) studies have showed reduced nuclear volume and condensed cytoplasm. The mRNA expression of apoptotic gene p53 and caspase 3 was up-regulated following ZnO nanoparticle exposure, which confirms the occurrence of apoptosis at the transcriptional level. Reactive oxygen species (ROS) was increased in a dose-dependent manner, and initiate lipid peroxidation of the liposomal membrane, which in turn regulate several signaling pathways and influencing the cytokinetic movements of cells. ZnO nanoparticles showed a dynamic cytotoxic effect in cervical carcinoma cells. ZnO nanoparticle might induce the apoptosis through increased intracellular ROS level. Moreover, up-regulated apoptotic gene expression confirms the occurrence of apoptosis. Taking all these data together, it may be concluded that ZnO nanoparticle may exert cytotoxicity on HeLa cell through the apoptotic pathway, implies the probable utility of ZnO nanoparticle in the cancer treatment and therapy.

1. Introduction

Nanomaterials have been widely used in the medicine, biology, and life science [1,2], and they are widely utilized in the drug development, drug carriers, and tracers [3,4]. Nanomaterials have been reported as toxic to mammalian cells, and can induce the apoptosis in tumor cells [5,6]. It was believed that nanoparticle elicits a greater biological response than microparticles [7], and it may affect the cellular activity [8]. Lynch et al. [9] pointed that the cytotoxicity of a nanoparticle due to its smaller size, high number per given mass, large specific surface area and generation of reactive oxygen species (ROS). The toxic metal has been widely used to treat some stubborn and severe diseases (Eczema, Psoriasis, and Acne) in the traditional Chinese medicine.

Apoptosis is a programmed cell death, in which cells will undergo a highly regulated program that plays a critical role in the prevention and treatment of cancer [10]. Cellular and nuclear shrinkage, nuclear fragmentation, condensation and formation of apoptotic bodies are believed to be key apoptotic features. Darzynkiewicz et al., have reported the condensation of cytoplasm and nucleus is known to produce membrane-bound apoptotic bodies that are phagocytized by macrophages [11]. The uncontrolled cell proliferation and loss of apoptosis are known to play a significant role in tumor formation [12]. The present study was aimed to investigate the cytotoxicity of ZnO nanoparticles against human cervical carcinoma cells.

2. Materials and Methods

2.1. Materials

Dulbecco's Modified Eagle's Medium (DMEM), dimethyl sulphoxide (DMSO), Sulforhodamine B (SRB) was purchased from Sigma-Aldrich (St. Louis, MO 63178 USA). Fetal bovine serum (FBS), penicillin–streptomycin and trypsin–EDTA were obtained from Welgene (Daegu, South Korea). Acridine Orange (AO), ethidium bromide (EB) and 2′,7′-dichlorofluorescein diacetate (DCFH-DA) were purchased from Santa Cruz Biotechnology, Inc. (Delaware Avenue, California, USA). Primers were purchased from Macrogen Inc. (South Korea).

2.2. Cell Culture

MDCK and HeLa cells were obtained from the Korean Cell Line Bank (South Korea). Cells were grown in growth medium supplemented with 10% FBS and 1% antibiotics (penicillin–streptomycin) in a CO_2 incubator under standard conditions.

* Corresponding author.
E-mail address: frenzram1980@gmail.com (D.H. Kim).

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2.3. Synthesis of ZnO Nanoparticles

ZnO nanoparticles were synthesized according to the method of Muthuraman et al., [13]. ZnO nanoparticles were synthesized by the addition of 6 g zinc acetate and 3 g polyvinyl alcohol and heated at 70 °C. The homogenous solution was prepared and heated at 120 °C to evaporate the solvent and to form a hard homogeneous gel. After that, the gel was pyrolyzed for 4 h at 400 °C. The polymeric network of polyvinyl alcohol was burnt via outer surface. The zinc acetate salt was calcinated and transformed into ZnO particles in the pyrolysis process.

2.4. Characterization of ZnO Nanoparticles

The prepared ZnO nanoparticle was characterized by scanning electron microscopy (SEM) and UV–visible spectrophotometer. The size and morphology of the ZnO nanoparticles were examined by SEM at a voltage of 20 KV (JEOL, JSM-6390). The UV–visible spectra of the particles were obtained with a visible/UV-spectrophotometer (Agilent Technologies, Cary 100 UV–Vis spectrophotometer).

2.5. SRB Assay

MDCK and HeLa cells were cultured at a density of 2×10^4 cells/well in a 96-well plate and allowed to adhere for 24 h. Then, the cells were treated with ZnO nanoparticle at different concentrations (0.001–0.07 mg/ml) for 48 h. The cytotoxic effect of ZnO nanoparticle on HeLa cells was determined by the SRB assay [14].

2.6. Morphological Observation by a Light Microscope

Cells were cultured in 6-well plates at a density of 2×10^5 cells/well. After 24 h adherence, the cells were treated with ZnO nanoparticle of different concentrations (0.02 & 0.03 mg/ml) for 48 h. At the end of treatments, the morphology of HeLa cells was examined by the light microscope (Nikon, Eclipse, 80i, Melville, NY 11747–3064, U.S.A).

2.7. Morphological Observation by a Fluorescence Microscope

Cells were cultured in 6-well plates at a density of 2×10^5 cells/well. After 24 h adherence, the cells were treated with ZnO nanoparticle of different concentrations (0.02 & 0.03 mg/ml). Cells were viewed under a fluorescence microscope (Axiovert 2000, Carl Zeiss, Germany) [15].

2.8. Confocal Laser Scanning Microscope (CLSM)

Cells were cultured in a confocal dish at a density of 2×10^5 cells/well. After 24 h adherence, the cells were treated with ZnO nanoparticle of different concentrations (0.02 & 0.03 mg/ml) for 48 h. Cells were processed and viewed immediately under CLSM ($1X81^R$ Motorized Inverted Microscope, Olympus [15].

2.9. mRNA Expression

Total RNA was isolated from the control and ZnO nanoparticle-treated samples [13]. The qPCR was performed using primers specific for p53 (forward: 5′-TAACAGTTCCTGCATGGGCGGC-3′, reverse: 5′-AGGACAGGCACAAACACGCACC-3′), caspase 3 (forward: 5′-TTAATAAA GGTATCCATGGAGAACACT-3′, reverse: 5′-TTAGTGATAAAAATAGAGTT CTTTTGTGAG-3′) and a housekeeping gene GAPDH (forward: 5′-GGTC ACCAGGGCTGCTTTT-3′, reverse: 5′-ATCTCGCTCCTGGAAGATGGT-3′). The relative ratios were calculated based on the $2^{-\Delta\Delta}{}_{CT}$ method [16].

2.10. Measurement of ROS Level

HeLa cells were seeded in 96-well plates at a cell density of 5000 cells/well. Cells were processed, and fluorescent images were obtained using a fluorescence microscope (Axiovert 2000, Carl Zeiss, Germany) [14].

2.11. Statistical Analysis

All experiments were done in triplicate, and all values were expressed as mean ± SD. The difference between control and treatment group was evaluated using ANOVA (SPSS 16). A $p < 0.05$ was considered statistically significant.

3. Results

3.1. Characterization of ZnO Nanoparticle

The UV–vis spectrum of the ZnO nanoparticles showed a wide absorption below 400 nm (Fig. 1A). The SEM images showed that the ZnO nanoparticle were all almost uniform in size (Fig. 1B). The diameter of the ZnO nanoparticle approximately 10 nm.

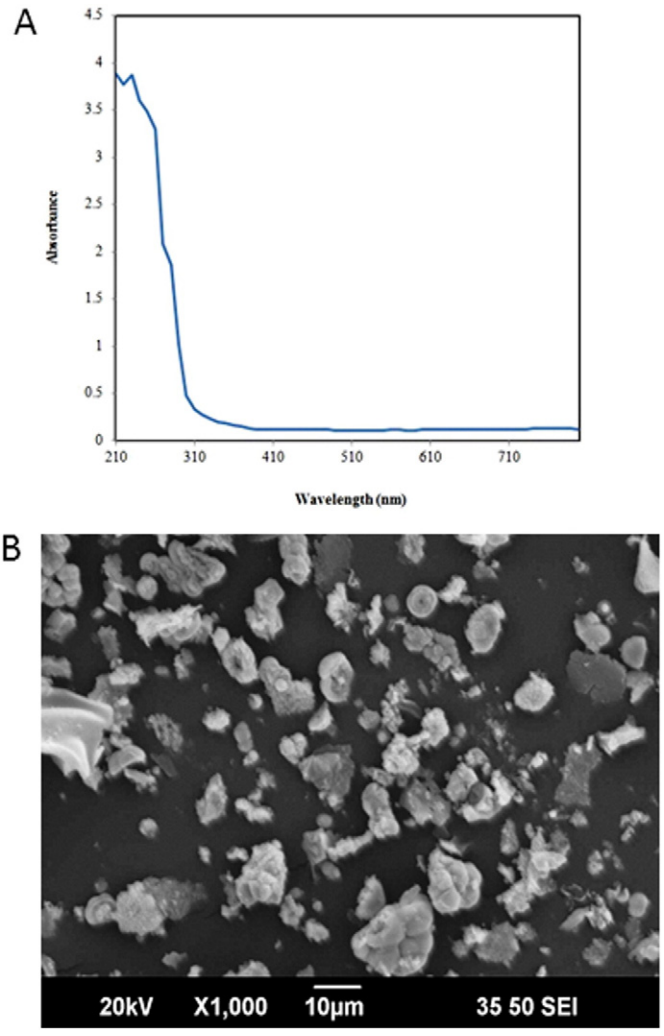

Fig. 1. UV–vis spectra of the ZnO nanoparticle. ZnO nanoparticles were dissolved in PBS, and PBS served as a reference (A). SEM images of the ZnO nanoparticle. ZnO nanoparticles were all almost uniform in size, and the mixture was homogeneous (B).