http://pmjournal.ir Original Article

Autumn 2020, Volume 5, Issue 19 (16-18)

Titanium Dioxide Nanoparticles can Induce Apoptosis in Cancer Cells

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DOI: 10.22034/pmj.2020.240047

Submitted: 2020/06/21
Accepted: 2020/07/29

Keywords:
Double staining
TiO2-NP
Hepatocellular
Apoptosis

Abstract

Nanotechnology involves the creation and manipulation of materials at the nanoscale to create products that exhibit novel properties. Titanium dioxide nanoparticles (TiO2-NPs), as one of the top five nanoparticles in annual production, are widely used in industries ranging from healthcare to drug delivery. The properties of TiO2-NPs have raised concerns regarding toxicity. Annexin V-FITC/PI double staining and a flow cytometer were used to detect apoptosis. A significant increase was observed in apoptosis at different concentrations of TiO2-NPs (50-100 µg/ml) varying between 25-40% apoptosis by 24 h. It is recommended that this study be performed in vivo to investigate the apoptotic and toxic effects of these substances further.

INTRODUCTION

Nanoparticles have wide applications in various fields due to their small size. Titanium dioxide nanoparticles (TiO2-NPs) are bright with a high refractive index (n=2.4), making them suitable for an industry dealing with toothpaste, pharmaceuticals, coatings, papers, inks, plastics, food products, cosmetics, and textile [1]. Therefore, potential widespread exposure may occur during both manufacturing and end-users. As an ultrafine sized material, TiO2-NPs can enter the human body through different routes, such as inhalation (respiratory tract), ingestion (gastrointestinal tract), dermal penetration (skin), and injection (blood circulation) [2]. The extensive uses in medical research and industrial applications highlight many routes for TiO2-NPs to enter into human bodies potentially [3]. With different physicochemical properties in size, shape, surface charge, and ligand, nanoparticles exhibit different biocompatibilities when interacting with different cells. Apoptosis is a common method of programmed cell death, characterized by depolymerization of the cytoskeleton, cell shrinkage, chromatin condensation, nuclear fragmentation, and transport phosphatidylserine to the cell surface [4]. Its cytological features include nuclear condensation and DNA fragmentation [5]. Nanoparticles can disrupt normal cellular function via cytotoxic stress and are responsible for membrane damage [6]. There are several genes and proteins reported to be involved in apoptotic pathways. Proteins of the Bcl-2 family comprise proapoptotic and antiapoptotic regulators of programmed cell death. The intended action mode of each component protein is to protect or destroy mitochondrial integrity, thereby activating or inhibiting the release of downstream factors, such as cytochrome C (Cyt C), thus resulting in the activation of caspase-3 [7]. The Bcl-2/Bax ratio determines whether cell survival or apoptotic cell death occurs and is an indicator of the strength of apoptosis [8]. Detection of apoptosis using these common markers can increase the confidence and accuracy of assays. The liver is the most important detoxifying organ so that it removes toxins from the circulation before excretion and is the target organ for numerous poisons [9]. Protecting the health of and alleviating the burden on the hepatic system is a key focus in life science research. There is evidence that TiO2-NPs can accumulate in the liver and cause severe damage following intravenous administration. In this study, the HL-7702 cell line was used as a model system to explore TiO2-NPs induced death of human hepatocytes and investigate the mechanisms involved.

METHOD AND MATERIALS

Commercial TiO2-NPs (P-25 type, 21 nm in average size) were provided. TiO2-NPs were suspended in RPMI 1640 cell culture medium and dispersed by an ultrasonic vibrator for 10 min.

The suspension was then characterized through DLS using a Zeta PALS+ BI-90 Plus at a wavelength of 659 nm. Human normal hepatocyte HL-7702 cells were purchased. HL-7702 cells were maintained in

RPMI-1640 medium, containing 10% fetal bovine serum (Biological Industries) with 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were incubated at 37°C in a 5% CO2 humidified atmosphere and continuously passaged every 2-3 days. Cells were seeded in culture plates at a density of 1x105 cells/ml for experiments. Following incubation for 24 h, cells were treated with TiO2-NPs dispersed in serum-free RPMI-1640 at concentrations of 0, 1, 10, 50, and 100 μg/ml at 37°C for 24 h. Cells maintained in RPMI-1640 without TiO2-NPs were used as the control group. Cells were stained by 5 μl Annexin V-FITC for 10 min and 5 μl PI for 5 min at room temperature in the dark, according to the protocols provided by the manufacturer of an Annexin V-FITC/PI Apoptosis Assay kit. The quantification of apoptosis induced by TiO2-NPs in HL-7702 cells was conducted via flow.

RESULTS

Annexin V-FITC/PI double staining and a flow cytometer were used to detect apoptosis. A significant increase in apoptosis was observed at concentrations of TiO2-NPs of 50-100 μg/ml, varying between 25-40% apoptosis by 24 h.

DISCUSSION

Nanotechnology involves the creation and manipulation of materials at the nanoscale to create products that exhibit novel properties. Nanomaterials ranging from 1 to 100 nm have been used to create unique nanosized devices possessing novel physical and chemical properties [10]. Because of these special properties, nanomaterials are widely used in many fields. TiO2-NPs, as one of the top five nanoparticles in annual production, are widely used in industries ranging from healthcare to drug delivery [11]. The properties of TiO2-NPs, such as the minute size, photocatalytic activity, and high biological reactivity, have raised concerns regarding toxicity [12].

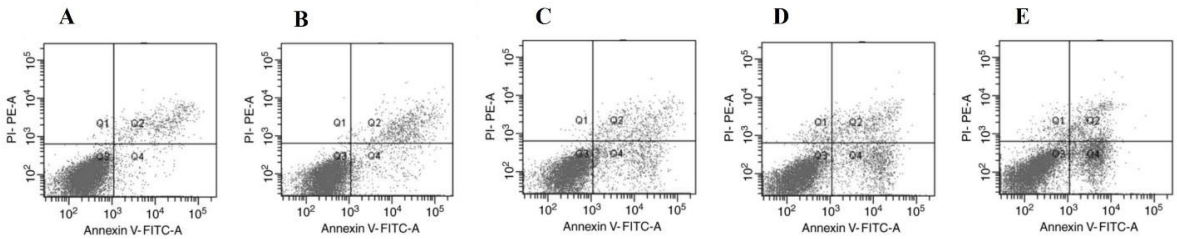

Fig.1. Scale bar indicates 10 µm. The four quadrants represent normal (C3), early apoptotic (C4), late apoptotic, or necrotic (C2), and mechanically damaged cells (C1). Annexin V-FITC/PI staining was conducted to assess cell apoptosis

Several studies reported that TiO2-NPs could cause damage to different cells such as human lymphoblastoid cells, syrian hamster embryo fibroblasts, and BEAS-2B cells [13]. The liver is the most important detoxifying organ that removes toxins from the circulation before excretion and is the target organ for numerous poisons. Protecting the health of and alleviating the burden on the hepatic system is a key focus in life science research [14]. In this study, the cytotoxicity of TiO2-NPs was assessed in HL-7702 cells cultured with different nanoparticle concentrations. Results showed a significant increase in apoptosis at different concentrations of TiO2-NPs (50-100 µg/ml) varying between 25-40% apoptosis by 24 h. It is recommended that this study be performed in vivo to investigate the apoptotic and toxic effects of these substances further.

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