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Investigation of the toxicity of bismuth oxide nanoparticles in various cell lines

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HIGHLIGHTS

- This is the first evaluation of toxicity profile of Bi_2O_3 nanoparticles in different cell lines.
- Bi_2O_3 nanoparticles decreased the cell viability by disrupting on mitochondria and lysosome.
- Bi_2O_3 nanoparticles induced oxidative damage in HepG2, NRK-52E and Caco-2 cells.
- Bi_2O_3 nanoparticles highly induced cell death.
- Bi_2O_3 nanoparticles should raise concern about their safety in consumer products.

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ABSTRACT

Nanoparticles have been drawn attention in various fields ranging from medicine to industry because of their physicochemical properties and functions, which lead to extensive human exposure to nanoparticles. Bismuth (Bi)-based compounds have been commonly used in the industrial, cosmetic and medical applications. Although the toxicity of Bi-based compounds was studied for years, there is a serious lack of information concerning their toxicity and effects in the nanoscale on human health and environment. Therefore, we aimed to investigate the toxic effects of Bi (III) oxide (Bi_2O_3) nanoparticles in liver (HepG2 hepatocarcinoma cell), kidney (NRK-52E kidney epithelial cell), intestine (Caco-2 colorectal adenocarcinoma cell), and lung (A549 lung carcinoma cell) cell cultures. Bi_2O_3 nanoparticles (~149.1 nm) were easily taken by all cells and showed cyto- and genotoxic effects. It was observed that the main cell death pathways were apoptosis in HepG2 and NRK-52E cells and necrosis in A549 and Caco-2 cells exposed to Bi_2O_3 nanoparticles. Also, the glutathione (GSH), malondialdehyde (MDA), and 8-hydroxy deoxyguanine (8-OHdG) levels were significantly changed in HepG2, NRK-52E, and Caco-2 cells, except A549 cell. The present study is the first to evaluate the toxicity of Bi_2O_3 nanoparticles in mammalian cells. Bi_2O_3 nanoparticles should be thoroughly assessed for their potential hazardous effects to human health and the results should be supported with *in vivo* studies to fully understand the mechanism of their toxicity.

1. Introduction

Nanoparticles are widely used because of their chemical, mechanical, optical, magnetic, and biological properties. Presently, a number of products in domestic, industrial, and biomedical applications on the world markets include various nanoparticles (EPA, 2007; Barillet et al., 2010a; Capasso et al., 2014). However, their wide applications have brought about serious public health problems through dermal absorption, oral ingestion, and inhalation of nanoparticles of various sizes and compositions (Elsaesser and Howard, 2011; Alarifi et al., 2013). It is well known that nanoparticles can penetrate through biological barriers and accumulate in different organs and cause toxic effects, such as oxidative stress, DNA damage, cell death, and morphological changes (Brooking et al., 2001; Singh et al., 2009; Barillet et al., 2010b; Arora et al., 2012; Chen et al., 2015).

Bi_2O_3 is one of the important metal oxides and used in microelectronic industry (Bandoli et al., 1996), sensor technology (Hyodo et al., 2000), optical coating (Schuisky and Hårsta, 1996), ceramic glass manufacturing (Pan and Ghosh, 2000), biomedicine (Taufik

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et al., 2011), painting (Patil et al., 2005), determining of heavy metal ions in drinking water, mineral water and urine (Pauliukaite et al., 2002), and medicine such as an astringent in a medical and topical creams (Periasamy et al., 2011). Recently, the applications of Bi-based nanoparticles have attracted the attention especially in biological sciences such as bioimaging (Rabin et al., 2006), biosensing (Ding et al., 2010), biomolecular detection (Wang et al., 2011), and X-ray radiosensitizing (Hossain et al., 2010).

The toxic effects of Bi-based compounds differ depending on organ systems. A number of toxic effects in humans have been attributed to Bi-based compounds: nephropathy, encephalopathy, osteoarthropathy, gingivitis, colitis, stomatitis, and hepatitis (Slikkerveer and de Wolff, 1989). There are only a few reports about the toxicity of Bi_2O_3 nanoparticles in mammalian cells (Thomas et al., 2012; Hernandez-Delgadillo et al., 2013; Liman, 2013). Therefore, the toxic effect of Bi_2O_3 nanoparticles on mammalian cells is not fully understood.

In the study, we aimed to assess the cellular toxicity and possible toxicity mechanisms of Bi_2O_3 nanoparticles. The study was designed to investigate their cytotoxicity, genotoxicity, oxidative damage, and apoptosis in HepG2 hepatocarcinoma, NRK-52E kidney epithelial, Caco-2 colorectal adenocarcinoma, and A549 lung carcinoma cells exposed to Bi_2O_3 nanoparticles. The assays and cell lines are both widely used for nanoparticle toxicity studies and might reflect the different exposure ways (Han et al., 1994; Brand et al., 2000; Leussink et al., 2002; Rached et al., 2008; Cavallo et al., 2015; Goya et al., 2015).

2. Material and methods

2.1. Chemicals

Cell culture mediums (Eagle's minimum essential medium (EMEM), Dulbecco's modified eagle medium (DMEM), DMEM F-12), foetal bovine serum (FBS), phosphate buffered saline (PBS 10x), and antibiotic-antimycotic solution were purchased from Wisent Bioproducts (Quebec, Canada). Bi_2O_3 nanoparticles (Cat. No. 631930), neutral red dye, ethylenediaminetetraacetic acid (EDTA), and MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). GSH, 8-OHdG, MDA, and PC enzyme–linked immune sorbent assay (ELISA) kits were purchased from Shanghai YeHua Biological Technology Co., Ltd. (Shanghai, China). Annexin V-FITC apoptosis detection kit with propidium iodide (PI) and protein assay dye reagent were purchased from BioLegend (San Diego, CA, USA). Bio-Rad (Munich, Germany) and all other chemicals were purchased from Merck (Kenilworth, NJ, USA).

2.2. Particle size characterization

Bi_2O_3 nanoparticles were suspended in both Milli-Q water and cell culture medium with 10% FBS. The suspensions were sonicated for 10 min and then dropped on carbon-coated, 200 mesh copper grid and allowed to dry prior to measurement by transmission electron microscopy (TEM) (JEM-2100 HR, JEOL, USA). The average diameter was calculated from measuring over 100 particles in random fields of TEM view.

2.3. Cell cultures and exposure conditions

The cell lines were obtained from American Type Culture Collection (ATCC) (Rockville, MD, USA). The following cell lines were chosen to evaluate the toxicity of Bi_2O_3 nanoparticles: HepG2 human hepatocarcinoma cells (ATCC® HB-8065), NRK-52E rat kidney proximal tubular epithelial cells (ATCC® CRL-1571), Caco-2 human colorectal adenocarcinoma cells (ATCC® HTB-37), and A549 human lung carcinoma cells (ATCC® CCL-185). The cells were incubated in suitable medium supplemented with FBS and antibiotic-antimycotic solutions at 5% CO_2, 90% humidity and 37 °C for 24 h (60–80% confluence) in CO_2 incubator (Heracell™ 150i, Thermo Scientific, Waltham, MA, USA) according to the ATCC's instructions. The cell densities were 1×10^4 cells/mL for cytotoxicity assays, 1×10^5 cells/mL for comet assay, 1×10^7 cells/mL for cellular uptake assay, and 1×10^6 cells/mL for both apoptosis/necrosis and oxidative damage assays.

Bi_2O_3 nanoparticles were suspended at 1 mg/mL concentration in cell culture medium with 10% FBS and sonicated at room temperature for 5 min to avoid the aggregation/agglomeration of nanoparticles before exposure. The particle suspensions were freshly prepared before exposure, diluted to appropriate concentrations with the culture medium, and then immediately applied to the cells. The exposure time to the particle suspensions was 24 h.

2.4. Cellular uptake assay

The uptake of nanoparticles into the cells at 100 μg/mL was evaluated by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Electron X series 2, Waltham, MA, USA) method. After exposure to the particle suspensions, the cells were washed several times with equal volumes of PBS and counted by LUNA™ cell counter (Logos Biosystems, Annandale, VA, USA). The cells were re-suspended in 6 M nitric acid and incubated at room temperature for 1 h, then stored at −20 °C until analysis. The acid-digested samples were assayed for Bi amount with ICP-MS. Also, Bi content of the unexposed cells for every cell line was measured.

2.5. Cytotoxicity assays

Cytotoxic activities of the Bi_2O_3 nanoparticles were determined by MTT and neutral red uptake (NRU) assays based on different cellular mechanisms depending on damaged region. The cells exposed final concentrations of 0.1–120 μg/mL of Bi_2O_3 nanoparticles. MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide, is a water-soluble, yellow-coloured salt reduced by the mitochondrial succinate dehydrogenase to insoluble purple formazan product. Mitochondrial succinate dehydrogenase is only active in viable cells. Therefore, in the MTT assay, colour changes by activity of the enzyme are used as a cytotoxicity endpoint (Van Meerloo et al., 2011). Neutral red is a weak cationic dye that accumulates in lysosomes by non-ionic passive diffusion and binds to anionic and/or phosphate groups of the lysosomal matrix by electrostatic hydrophobic bonds. In NRU assay, lysosomal integrity is used as an indicator of cell viability by up taking neutral red dye into cells (Repetto et al., 2008). Optical density (OD) was read at 590 nm and 540 nm for MTT and NRU, respectively, using an Epoch microplate spectrophotometer system (BioTek, Winooski, United State). In every assay, triton X-100 (1%) and PBS were used as positive and negative controls (unexposed cells), respectively. The inhibition of enzyme activity in cells was calculated as compared to negative control. The results were expressed as the cell death (%) and the half maximal inhibitory concentration (IC_{50}).

2.6. Genotoxicity assay

Genotoxic activities of the Bi_2O_3 nanoparticles were determined by comet assay. The cells exposed final concentrations of 0.1–30 μg/mL of Bi_2O_3 nanoparticles. The assay principle is to measure DNA strand breaks in eukaryotic cells lysed with detergent and high salt and embedded in agarose on a microscope slide. Hydrogen peroxide (H_2O_2) (50 μM) and PBS were used as positive and