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CYP1A and POR gene mediated mitochondrial membrane damage induced by carbon nanoparticle in human mesenchymal stem cells

Ali A. Alshatwi[a,*], Vaiyapuri Subbarayan Periasamy[a], Pandurangan Subash-Babu[a], Mohammed A. Alsaif[b], Abdulrahman A. Alwarthan[c], Lei K.A.[d]

[a] Nanobiotechnology and Molecular Biology Research Lab, Dept. of Food Science and Nutrition, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia
[b] College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia
[c] Dept. of Chemistry, College of Science, King Saud University, Riyadh, Saudi Arabia
[d] Dept. of Nutrition and Food Science, University of Maryland, College Park, MD 20742, USA

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ABSTRACT

Nanoparticles (NPs) can cause respiratory and cardiovascular problems, furthermore small carboxyl polystyrene NPs induce hemolysis, activate platelets and induce inflammation in human blood. Carbon nanoparticles (CNPs) are known to interfere with cellular metabolism, specific cellular functions and moreover may cause cellular toxicity. We aimed to study the influence of CNPs on oxidative stress, mitochondrial membrane damage and intracellular gene expression in human mesenchymal stem cells (hMSCs). CNPs cause a dose and time dependent growth inhibition in hMSCs at a dose range from 50 to 400 μg/mL. Exposure of CNPs toxic doses viz., 50 μg/mL (D1) and 100 μg/mL (D2) decreased intracellular mitochondrial membrane potential compared to control. CNPs treated cells were found to lose their morphology due to cell membrane damage have been confirmed by propidium iodide staining and fluorescence microscopic analysis. Oxidative stress responsive genes like GSTM3 and GSR1 expression have increased a fold when compared to control, interim there is no change were observed in SOD and GPx. We found an increased expression of CYP1A and POR genes by at least 2- fold, which is involved in mitochondrial trans-membrane potential. In conclusion, routine and high exposure of CNPs to hMSCs increased oxidative stress and mitochondrial membrane damage.

1. Introduction

Nanoparticle research holds a potential in the areas such as diagnostics, cancer detection and targeted drug delivery. However their safety level has been subject to an issue, particularly through the discovery of toxic nanoparticles. The reactiveness of nano-sized particles is difficult to assess, so properties are still relatively unknown. The size of these new nanoparticles (<100 nm) with their high surface area and unusual surface chemistry and reactivity poses unique problems for biological cells and the environment (Panessa-Warren et al., 2006).

* *Corresponding author at*: Department of Food Science and Nutrition, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2460 Riyadh 11451, Saudi Arabia. Tel.: +996 1 467 7122; fax: +996 1 467 8394.
E-mail address: alialshatwiksu@gmail.com (A.A. Alshatwi).
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Carbon nano materials formed as a result of combustion processes arising in nature from volcanoes and forest fires have been present in the earth's atmosphere for at least 10,000 years in the form of carbon nanotubes (CNTs) and nanocrystals (Murr et al., 2005). While the inhalation of ultrafine particles may contribute to an increase in mortality and morbidity (Oberdorster et al., 2005), the effects of cells and following skin exposure are limited.

CNTs have been intensively studied for their extraordinary electronic and mechanic properties point toward a great variety of potential future applications (The Royal Society, 2004). The development of nanotechnologies leads to considerable concern regarding the potential biological effects and human toxicity of nanomaterials (Oberdorster et al., 2005). Carbon block (CB) is one of the substantial health and environmental risks and are produced in huge quantities (Donaldson et al., 2005; Unfried et al., 2008). Epidemiological as well as experimental studies have confirmed the role of CB-NPs in aggravating pulmonary disorders such as asthma as well as lung cancer, pulmonary fibrosis and systemic cardiovascular disorders (Donaldson et al., 2005). They are also used as food additives, in sunscreens, water cleanup technology, white pigments for paints, papers, plastics and ceramics. CB has been reclassified as a possible human carcinogen by the International Agency for Research on Cancer (IARC, 2006).

Topical applications and consumption of NP formulations are accepted either a pharmacological agent or nutritional value by majority of population, which creates a health alarm for the public (Khundkar et al., 2010). The reason behind this seems to be their potential effect on human health following consumption, which has received little attention; and the lack of awareness, which has raised concerns regarding the safety of nanomaterials in biological and food applications. Recent reports have confirmed that the presence of carbon nanoparticles in food caramels, such as jaggery, sugar caramel, corn flakes and biscuits (Md Palashuddin et al., 2012). There is an immediate attention need to monitor the particle sizes, dose, temperature-dependent formation of food nanoparticles and cellular and molecular toxicity of carbon nanoparticles will be determined.

Recent advances in nanotechnology have increased the development and production of many new nanomaterials with unique characteristics for industrial and biomedical uses. Nanoparticles have also been known to pass through cell membranes and cause possible harm to healthy cells. However, the in vitro cytotoxicity assessment of carbon-based nanomaterials is problematic due to the adsorptive nature. As a result of these conflicting reports concerning CNPs cytotoxicity, it is necessary to evaluate the systematic cytotoxicity assays in different cells. We have chosen human mesenchymal stem cells (hMSCs) for our present study, because hMSCs derived from adult bone marrow have the tendency to differentiate into a specific type of progenitor cells. In this present study, hMSCs have been used to analyze the intracellular toxicity of carbon nanoparticle followed by cytotoxic assay, florescence microscopic analysis for mitochondrial damage, nuclear and cellular damage and oxidative stress responsive gene expression analysis.

2. Materials and methods

2.1. Materials

Carbon nanoparticles (CNPs) (<50 nm) and Propidium Iodide (PI) were purchased from Sigma–Aldrich (St. Louis, MO, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl benzimidazolyl carbocyanine iodide (JC-1), dimethyl sulfoxide (DMSO) and fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA, USA). Mesencult® mesenchymal stem cell (MSC) basal medium was procured from Thermo Scientific Hyclone (USA). The QuantiTect Primer assay, FastLane Cell cDNA Kit and QuantiFast SYBR Green PCR Kit were obtained from QIAGEN (Germany). All the other chemicals used were of research grade.

2.2. Cell culture

The hMSCs was obtained from Thermo Scientific Hyclone (USA). The cells were cultured in Mesencult® mesenchymal stem cell (MSC) basal medium with MSC stimulatory supplements (Human), CNP, 100 U/mL penicillin and 100 μg/mL streptomycin in 96-well culture plates at 37 °C in a humidified atmosphere containing 5% CO_2. All experiments were performed using cells from passage 15 or less.

2.3. Cell viability assay

The cytotoxicity of CNPs (<50 nm) was analyzed on solution containing doses ranging from 25, 50, 100, 200 and 400 μg/mL using MTT assay (Blagosklonny and El-Diery, 1996). The CNPs were prepared at different concentrations in growth medium and were added to the wells 24 h after hMSCs seeding of 1×10^4 cells per well in 200 μL of fresh culture medium. After incubation, 20 μL of MTT solution (5 mg/mL in phosphate-buffered saline (PBS) were added to each well. The plates were wrapped with aluminum foil and incubated for 4 h at 37 °C. The plates were centrifuged and purple formazan product was dissolved by the addition of 100 μL of DMSO to each well. The absorbance was monitored at 570 (measurement) and 630 nm (reference) using a 96-well plate reader (Bio-Rad, CA, USA). Data were collected for three replicates each and used to calculate the mean. The percentage of toxicity was calculated, from this data, using the formula:

$$\frac{\text{mean OD untreated cells} - \text{mean OD of treated cells}}{\text{mean OD of untreated cells}} \times 100$$

2.4. Microscopic studies

The particle uptake was analyzed using bright-field microscopy after 24 h in hMSCs treated with CNPs. The nuclear morphology of hMSCs was analyzed after treatment with CNPs for 24 h. Control cells were grown in the same manner in the absence of CNPs. The cells were trypsinized and fixed with ethanol. Then, cell nuclei were stained by treatment with 1 mg/mL propidium iodide at 37 °C for 15 min