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Altered global gene expression profiles in human gastrointestinal epithelial Caco2 cells exposed to nanosilver

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ABSTRACT

Extensive consumer exposure to food- and cosmetics-related consumer products containing nanosilver is of public safety concern. Therefore, there is a need for suitable *in vitro* models and sensitive predictive rapid screening methods to assess their toxicity. Toxicogenomic profile showing subtle changes in gene expressions following nanosilver exposure is a sensitive toxicological endpoint for this purpose. We evaluated the Caco2 cells and global gene expression profiles as tools for predictive rapid toxicity screening of nanosilver. We evaluated and compared the gene expression profiles of Caco-2 cells exposed to 20 nm and 50 nm nanosilver at a concentration 2.5 μg/ml. The global gene expression analysis of Caco2 cells exposed to 20 nm nanosilver showed that a total of 93 genes were altered at 4 h exposure, out of which 90 genes were up-regulated and 3 genes were down-regulated. The 24 h exposure of 20 nm silver altered 15 genes in Caco2 cells, out of which 14 were up-regulated and one was down-regulated. The most pronounced changes in gene expression were detected at 4 h. The greater size (50 nm) nanosilver at 4 h exposure altered more genes by more different pathways than the smaller (20 nm) one. Metallothioneins and heat shock proteins were highly up-regulated as a result of exposure to both the nanosilvers. The cellular pathways affected by the nanosilver exposure is likely to lead to increased toxicity. The results of our study presented here suggest that the toxicogenomic characterization of Caco2 cells is a valuable *in vitro* tool for assessing toxicity of nanomaterials such as nanosilver.

1. Introduction

An exponential increase in the use of nanomaterials in consumer products has been reported in recent years. The human exposure to these products is rapidly expanding and, therefore, their potential for adverse health effects is of concern. The silver nanoparticles show a wide spectrum of antibacterial and antifungal properties [20,42,52]. Therefore, nanosilver is one of the most commonly used nanomaterials in consumer products. However, in spite of their widespread use, very limited information is available on their potential toxicity. Use of nanosilver in food, food contact materials, and dietary supplements has significantly increased in recent years [53]. Thus, the use of food-related nanosilver is highly relevant for human exposure [53], and its toxicity screening is necessary to better ensure the consumer safety.

Ingestion is a major route of human exposure of food-related nanoparticles. It has been reported that the nanoparticles present in gastrointestinal tract play an important role in the development of colon disease [54]. The epithelium of the small intestine and colon provide protection against toxicants in the blood stream [55]. The epithelial cells separate the gastrointestinal tract from the systemic circulation and prevent the uptake of toxicants from the bloodstream. Injury to these epithelial cells impairs their protective function.

Animal studies required for toxicity screening are costly and time consuming. Therefore, the search for suitable *in vitro* models to accurately predict toxicity *in vivo* is of interest. One of the important requirements of safety evaluation of a potential toxicant is its reliable and reproducible toxicity information. The costly and time consuming animal studies and the lack of human data led to the evaluation of alternative *in vitro* models for reliable and reproducible mechanistic information that can be used for risk assessment. Human cell lines in culture are sensitive tools for high-throughput toxicity screening and they have the potential to reduce the use of animals for toxicological testing in the 21st century [27].

The human gastrointestinal epithelial Caco2 cells are widely used as an *in vitro* model for traditional chemical toxicity testing, representing the oral route of exposure. They are well characterized

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and show many of the morphological and biochemical characteristics of small intestine enterocytes [56,57]. Recently, [61] have demonstrated that the differentiated Caco2 cells closely resemble the physiological intestinal epithelium. [58] have used the Caco2 cells as an *in vitro* model for the prediction of intestinal drug absorption *in vivo*. Therefore, the Caco2 cells have been successfully used as a useful *in vitro* screening tool for toxicity evaluation of compounds [59,60]. Therefore, in this study we evaluated the Caco2 cells to determine if they can be used as an *in vitro* model for predictive rapid screening of food-related nanomaterials.

Multicellular organisms react to environmental changes primarily at the cellular level. Genomic responses to toxic exposures offer valuable tools for toxicity evaluation of potential toxicants [62]. The gene expression profiles provide molecular information on the cell-toxicant interactions. The cells respond to environmental stress through adaptive stress response pathways. These pathways are activated at significantly lower toxicant concentrations than those causing cellular injury detected by conventional biochemical methods [62]. The toxicogenomic effects of nanoparticles at low levels especially below the detection limits of traditional biochemical endpoints of toxicity are unknown. Therefore, molecular biomarkers such as gene expression changes which can detect cellular injury at low levels of toxic exposure are of importance. Toxicogenomic endpoints are very useful for detection of early stages of toxicity that cannot be detected by conventional endpoints. Toxicogenomics shows subtle changes in gene expression. Identification of differentially expressed genes reveals the molecular mechanisms of toxic exposures. This technology uses DNA microarrays to evaluate the effects of potential toxicants at the molecular level. It allows the analysis of expression of thousands of genes simultaneously. Toxicogenomic techniques and human cells *in vitro* have the potential to reduce the use of animals and to eliminate the need for interspecies extrapolation [27].

Recently we have reported the cytotoxic and genotoxic effects of 20 nm and 50 nm nanosilver in HepG2 and Caco2 cells [34–36,38] and toxicogenomic effects of these nanosilvers in HepG2 cells [37]. The purpose of the current study presented here was to determine if the Caco2 cells and the global gene expression profiles can be used as useful tools for predictive rapid toxicity screening of food-related nanomaterials such as 20 nm and 50 nm nanosilver.

2. Materials and methods

2.1. Materials

The 20 nm and 50 nm BioPure® silver nanoparticle citrate solution was purchased from nanoComposix (San Diego, CA). The human colon carcinoma Caco2 cells (ATCC HTB-37), were obtained from the American Type Culture Collection (ATCC), Manassas, VA. Deep-frozen vials of stock cells were routinely stored in liquid nitrogen freezer. Dulbecco's modified Eagle's medium (DMEM) GlutaMax, Hanks' balanced salt solution (HBSS), HEPES, phosphate buffered saline (PBS), trypsin-EDTA solution and 0.4% trypan blue solution were purchased from Invitrogen Corporation (Grand Island, NY). Fetal bovine serum (FBS) was purchased from the Hyclone Labs (Logan, UT). The sterile nonpyrogenic polystyrene cell culture flasks and plates were purchased from Corning (Corning, NY) and Becton-Dickinson (Franklin Lakes, NJ), respectively. All other chemicals were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Buffer RLT, QIAshredder spin column and EZ1 RNA Cell Mini Kit were purchased form Qiagen (Valencia, CA). The RNA 6000 Nano Reagent Kit was purchased from Agilent (Santa Clara, CA). The Affymetrix GeneChip 3′ IVT Express Kit was purchased from Affymetrix (Santa Clara, CA).

2.2. Methods

2.2.1. Characterization of nanoparticles

The silver nanoparticles were characterized by the dynamic light scattering (DLS), transmission electron microscopy (TEM) and inductively coupled plasma-mass spectrometry (ICP-MS) analysis as described previously [34,37]. The stock solution of the nanosilver in aqueous 2 mM citrate was stored at 4 °C in small aliquots. The desired concentrations of silver nanoparticles for cell exposures were prepared fresh by diluting the stock solution with the cell culture medium just before the experiment [63–65,34,35].

2.2.2. Cell culture

Human colon carcinoma Caco2 cells were stored routinely in small aliquots in liquid nitrogen and the experimental cultures were prepared from the frozen stock cells and always kept in a subconfluent state [66,67,34,35,37]. The cells were cultured in 75 cm^2 culture flasks in Dulbecco's modified Eagle's medium (DMEM) with GlutaMax containing 1.5% glucose and supplemented with 10% fetal bovine serum, 1% MEM non-essential amino acids and 10 mM HEPES buffer [67–70,9,34,35,37] in a saturating humidified atmosphere of 5% CO_2 in air at 37 °C. The culture medium was changed every 3–4 days. The cultures were used for testing within 10 passages after the cells were received from the ATCC.

2.2.3. Cytotoxicity assay

The cytotoxicity of 20 nm and 50 nm nanosilver was determined fluorometrically by the resazurin (Alamar Blue) reduction assay as described previously [34,37]. Briefly, cells were seeded in 96-well plates and treated with varying concentrations (0.5–25.0 μg/ml) of the 20 nm or 50 nm nanosilver, or with the vehicle control (cell culture medium), for 4 h or 24 h at 37 °C, washed with sterile HBSS, and then incubated with resazurin for 30 min at 37 °C in a plate reader using the Sigma Resazurin Assay kit. The rate of increase of resorufin fluorescence was measured at 545 nm excitation and 590 nm emission. Each concentration was tested with ten replicates. Statistical analysis of the results was conducted using one-way ANOVA.

2.2.4. Treatment of cells with nanosilver

When the cells were grown to approximately 70–80% confluence, they were prepared for the experimental procedures. The cells were washed with Ca- and Mg-free HBSS and harvested from the 75 cm^2 culture flasks by 0.05% trypsin-EDTA. A single cell suspension in the culture medium was obtained by repeated trituration. Cell counts and cell viability were determined by trypan blue dye exclusion using a hemocytometer. A single cell suspension in the culture medium at an approximate density 1×10^5 cells/ml was prepared by serial dilution and this stock cell suspension was used for seeding cells for the study. The cells were seeded in 6-well plates (3 ml/well) for 24 h before treatment.

On the day of cell exposure the dosing solutions of 20 nm silver were prepared by serial dilutions of the stock solution in the cell culture medium immediately before use. The logarithmically growing cells were treated with the test agents. The cells were washed once with HBSS and the dosing solutions were added to the cells. The concentrations of the dosing solution and the time of exposure were selected to induce minimum toxicity. The cells were exposed to the nanosilver at a concentration of 2.5 μg/ml for 4 h or 24 h at 37 °C in a saturating humidified atmosphere of 5% CO_2 in air. The control cells received an equal volume of the vehicle (cell culture medium). After exposure the cells were washed with HBSS and prepared for the global gene expression profile analysis by DNA microarray.