The Effects Of Cerium Oxide Nanoparticles On The Pluripotent Cell Line P19

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Citation

S Cruz, J Watkins, E Johnican, Q Chen. *The Effects Of Cerium Oxide Nanoparticles On The Pluripotent Cell Line P19*. The Internet Journal of Genomics and Proteomics. 2020 Volume 8 Number 1.

DOI: 10.5580/IJGP.53614

Abstract

Nanoparticles (NPs) have a wide range of biological, medical and industrial applications. The rapid development of NPs has brought attention to the advancements it brings to the global society but has also raised major concerns around the adverse effects NPs pose to the environment and human health. One nanoparticle in particular cerium oxide (CeO2) is widely used in a variety of applications, for example, in 2001 it was reported that CeO2 nanoparticle emission would be around 22 million pounds. This increase in the release and availability of CeO2 NPs into the environment has brought on an interest in the evaluation of these NPs in biological systems is meaningful and pertinent to human health. The elucidation of the positive and negative benefits of CeO2 NPs appears to be inconclusive. CeO2 nanoparticles have been shown to have antioxidant properties. In contrast, studies have explained how CeO2 NPs generate oxidative stress and induce apoptosis in human lung epithelial cells. These findings on CeO2 NPs indicate cytotoxic and potential genotoxic effects upon cellular internalization. It has been reported that CeO2 NPs of various sizes show significant toxic effects on E. coli, human hepatoma and PC12 cells, related to oxidative stress. However, there are opposing scientific findings that have claimed that CeO2 NPs of small sizes do not cause any adverse effect but can protect cells from harmful effects of radiation and oxidative stress. There are numerous distinct elements that must be evaluated to truly determine the effects of nanoparticles, such as toxicity and the proteomic effects. In this study we hypothesize P19 pluripotent stem cells exposed to cerium oxide nanoparticles will modulate cellular changes in protein profiles. This study evaluated the protein profiles of P19 pluripotent stem cells exposed to cerium oxide nanoparticles utilizing 2D-Gel electrophoresis.

INTRODUCTION

Nanotechnology has been advancing at an infinite pace and also their applications in industrial and biomedical arenas have raised concerns about the adverse effects of nanoparticles (NPs) on human health and also the environment. Any object within the range of 1-100nm is by definition a nanomaterial. Nanomaterials include but are not limited to nanogels, nanofibers, nanotubes and other nanoparticles (rods, cubes, and spheres) [1, 2]. The materials from which NPs are often derived from, ranges from polymers, metals, carbon, silica, and materials of biological origin like lipids or acid. NPs have assortment of bio-reactive and or physicochemical properties. Metal oxide NPs are utilized in sunscreens, food, paints, textile, electronics, and biomedical applications and in imaging [3, 4, 7]. Studies declared that the value of engineered nanoparticles will increase to more than 20–30 billion dollars [5]. Engineered NPs are appealing for medical purposes thanks to their trans-locational properties into tissue with their small NPs surface to volume ratio it enables them to be adsorbed and carry therapeutic compounds readily [6]. The interest in engineered NPs has raised concerns over the unexpected harmful health effects due to nanotoxicity, which is creating a lag in the application of NPs. In the past our experienced with abestose and its exposure to our ecosystem and human health have identified the negative potential of nanoparticles. The understanding of mercury toxicity in fish has resulted in an acceptance of a decrease intake of top consumer fish types. For instance, recently a study has revealed that manufactured polystyrene NPs are transported from algae through zooplankton to fish which then affects the lipid metabolism and behavior of the consumer. The accumulation of nanometals after consumption in woman may cause harmful damage to a developing fetus. There is very little known of human exposure and also the toxicity related to engineered NPs, hence investigations on the biokinetics of assorted NPs in organisms is given much

prudence for information on absorption, metabolism, distribution and excretion of NPs.

Cerium oxide (CeO2) NPs are widely used in a variety of applications such as fuel cells, glass polishing agents, solar cells, ultraviolet absorbents, gas sensors and television tubes [8–11]. In an effort to reduce ignition temperatures of carbonaceous diesel exhaust particles and emission of particulate matter of diesel engines, CeO2 NPs have been employed as fuel additives [12]. This addition results in the emission of CeO2 NPs straight into the environment with unknown consequences. In 2001 it was reported that CeO2 NP emissions would be around 22 million pounds or more after this addition [12]. This increase in the release and availability of CeO2 NPs in the environment and the evaluation of these NPs in biological systems is meaningful and pertinent to human health [13]. It is important to note that human intake of NPs can be occupational exposure or environmental by inhalation or ingestion. Because of the inability to effectively absorb CeO2 NPs through the intestine, inhalation is the most direct way to gain exposure and the respiratory is a deposit site for NPs of varying sizes.

The elucidation of the positive and negative benefits of CeO2 NPs appears to be inconclusive. Studies on CeO2 NPs have shown biomedical applications as protectants against radiation induced damage and neurodegeneration and anti-inflammatory and antioxidant activity [14–19], but the assessment of genotoxicity was not performed. It is important to note that the antioxidant properties of CeO2 NPs are thought to be the results of the dual oxidation state CeO2 NPs or the pH value of cellular compartments where the NPs internalize [19, 28]. In contrast, studies have explained how CeO2 NPs generate oxidative stress and induce apoptosis in human lung epithelial cells [20, 21]. So, CeO2 NPs may indicate cytotoxic and genotoxic effects upon cellular internalization. It has been reported that CeO2 NPs of various sizes show significant toxic effects on E. coli, human hepatoma and PC12 cells, related to oxidative stress [22, 23]. In parallel scientific findings have claimed that CeO2 NPs of small sizes do not cause any adverse effect but can protect cells from harmful effects of radiation and oxidative stress, but this protection was cell type specific [24, 25]. Results from in vivo studies have shown inhalation of CeO2 NPs induces acute pulmonary and systemic toxicity in mice and rats in lieu of pro-inflammatory responses [26, 27]. Thus the conflicting results keep the toxicity endpoints relevant to human health elusive and they need to be addressed. It is therefore prudent to conduct a study to understand the comprehensive molecular mechanism of the toxicity of CeO2 NPs.

In this study the determination of the effects of cerium oxide nanoparticles on the profile of proteins in the P19 embryonal carcinoma cell line, a pluripotent stem cell line that divides rapidly and maintained easily [29]. In a cell proteins are crucial to the function of biological systems and dictate molecular and metabolic activities. Hence it is important that the scientific community understands as much as possible concerning how proteins change with relationship to abundance, expression, modification, compartmentalization and scaffolding. In order to peer into the changes of protein profiles of P19 cells exposed to cerium oxide, a proteomic approach will be undertaken. A proteomic approach such 2D-gel electrophoresis allows for a comprehensive examination of proteins. It is a widely used technique in proteomics that allows for the separation of proteins in complex samples using the isoelectric point value and relative molecular weight properties of proteins. This method easily allows for the identification of unique changes in protein profiles amongst treated and untreated sample populations. Protein profiling is continuously expanding and have been crucial in the identification of unique protein events in cancers and the development of treatments [30]. The use of proteomics in the study of the effects of nanoparticles in biological systems has been demonstrated by Pan et al. In this study they identified that metabolic processes and catalytic activity were the main biological processes and molecular functions effected in the lung tissues of BALB/c mice in response to 24 hour and 28 day exposures to zinc oxide nanoparticles. The research demonstrated a total of 18 proteins at 24 hours and 14 proteins at 28 days that had dramatically changed in the presence of zinc oxide [31]. In addition, a study was performed by Zhang et al. which showed that nanotparticles of different sizes and surface properties have varying effects on the accumulation of nanoparticles by cells, protein function and regulation of metabolism [32].

In the proliferation of cells definitive protein changes occur that regulate a number of molecular and metabolic processes, this study aims to identify and expand the understanding of the unique protein profile changes associated with P19 cells exposed to cerium oxide. In this study P19 cells untreated and exposed to a 25ug/ml concentration of cerium oxide, were grown for 24 hours. This time point and concentration was utilized as it showed an increase in cell proliferation that was significant.

METHODOLOGY

Cell culture

P19 cells were maintained in complete medium (alpha modified MEM, 2.5% fetal bovine serum (FBS) and 1% penicillin-streptomycin) at 37°C in a 5% CO2 atmosphere. The cultures were passaged every 2-3 days at 1:20 to 1:30 dilution.

Protein isolation and profiling

Cells exposed to 25 g/ml concentration of cerium oxide nanoparticles for 24 hours were analyzed by comparative 2D gel electrophoresis. Two-dimensional electrophoresis was performed according to the carrier ampholyte method of isoelectric focusing (O'Farrell, P.H., J. Biol. Chem. 250: 4007-4021, 1975, Burgess-Cassler, A., Johansen, J., Santek, D., Ide J., and Kendrick N., Clin. Chem. 35: 2297, 1989) by Kendrick Labs, Inc. (Madison, WI) as follows: Isoelectric focusing was carried out in glass tubes of inner diameter 3.3 mm using 2% pH 3-10 Isodalt Servalytes (Serva, Heidelberg, Germany) for 20,000 volt-hrs. One µg (Coomassie) or one hundred ng (silver) of an isoelectric focusing internal standard, tropomyosin, was added to each sample. This protein migrates as a doublet with lower polypeptide spot of MW 33,000 and pI 5.2. The enclosed tube gel pH gradient plot for this set of Servalytes was determined with a surface pH electrode. (O'Farrell, 1975) (Burgess-Cassler, Johansen, Santek, Ide, & Kendrick, 1989)

After equilibration for 10 min in buffer "O" (10% glycerol, 50 mM dithiothreitol, 2.3% SDS and 0.0625 M tris, pH 6.8), each tube gel was sealed to the top of a stacking gel that overlaid a 10% acrylamide slab gel (1.0 mm thick). SDS slab gel electrophoresis was carried out for about 5 hrs at 25 mA/gel. The following proteins (MilliporeSigma) were used as molecular weight standards: myosin (220,000), phosphorylase A (94,000), catalase (60,000), actin (43,000), carbonic anhydrase (29,000), and lysozyme (14,000). These standards appear along the basic edge of the silver-stained (Oakley, B.R., Kirsch, D.R. and Moris, N.R. Anal. Biochem. 105:361-363, 1980) or Coomassie Brilliant Blue R-250-stained 10% acrylamide slab gels. Gels were dried between sheets of cellophane paper with the acid edge to the left. (Oakley, Kirsch, & Morris, 1980)

Comparisons of Patterns

Each of the gels were overlaid with a transparent sheet for labeling polypeptide spot differences without marking the original gel. Two experienced analysts compared the protein pattern from normal P19 cell pellets with the protein pattern from treated P19 cell pellets. Polypeptide spots that were unique to or relatively darker in the gels from sample treated P19 cell pellets were outlined in black, while spots unique to or relatively darker in sample normal P19 cell pellets were outlined in red.

RESULTS

When looking at the normal untreated P19 cells at the 24-hour time point stained in silver and coomassie blue there are six protein spots identified. Compared to the P19 cells treated with 25 g/ml of cerium oxide nanoparticles at the 24-hour time point stained in silver and coomassie blue there are twenty-six protein spots identified. Protein spots that were unique to or relatively darker in the gels from sample treated P19 cell pellets were outlined in black, while spots unique to or relatively darker in sample normal P19 cell pellets were outlined in red. Together silver stain and coomassie blue stains were used to have a true representation of the proteins, since when only using silver stain some of the protein spots may become distorted due to the stain, but coomassie blue does not distort or begin to blend. Permitting proper visualization of the protein spots produced. The gel did not resolve high molecular weight proteins well, and the spots are not visible on the silver-stained gel. These spot differences were able to be verified on the Coomassie blue-stained gel.

Approximately 200 protein spots were identified with the molecular weight range of 20-220 kDa and between a pH range of 3 and 8, although more proteins may have been present, the protein spots that were counted were identifiable. Of the approximately 200 protein spots that were identified, 9 were found to be unique to the normal untreated P19 cells and 26 were found to be unique to the treated P19 cells. The unique protein spots identified in the normal untreated P19 cell gel were in the molecular weight range of 94-220 kDa and pH 5-7, while the unique protein spots identified in the treated P19 cell gel were in molecular weight range of 20-70 kDa and pH 4-7. Refer to Table 1 and Figure 1 for supporting data.

Figure 1

Silver-stained isoelectric focusing gel comparing the six unique protein spots of the normal P19 cells within the molecular weight range of 94-220 kDa and pH 5-7, with 26 unique protein spots of the treated P19 cells (red circles) and 9 unique spots for the control P19 cells (green circles) within the molecular weight range of 20-70 kDa and pH 4-7.

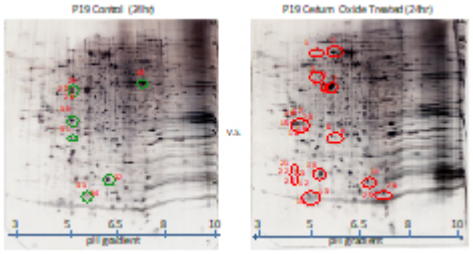

Table 1

Differential comparison of Treated P19 versus Control P19 Cells. Table 1. A total of 35 unique spots were identified and show differential spot intensity between control P19 versus treated P19 cells. There were 26 unique protein spots for the treated P19 cells and 9 unique spots for the control P19 cells within the molecular weight range of 10-90 kDa and pH 4-7. This table displays pI, Molecular Weight (MW), and spot intensity as either increased or decreased.

			Spot intensity	Spot intensity
Spot #	~MW(Kda)	~pI	P19 Control	Treated P19 (25ug/mL Cerium Oxide)
1	90	5		Increased
2	90	5.5		Increased
3	50	5		Increased
4	43	5		Increased
5	43	5		Increased
6	40	5.5		Increased
7	43	5.5		Increased
8	22	5.5		Increased
9	22	5.5		Increased
10	15	5		Increased
11	14	6.5		Increased
12	10	5		Increased
13	10	5		Increased
14	10	5		Increased
15	29	4.5		Increased
16	29	4.5		Increased
17	29	4.5		Increased
18	29	4.5		Increased
19	29	4.5		Increased
20	29	4.5		Increased
21	15	4.5		Increased
22	14	4.5		Increased
23	14	4.5		Increased
24	13	4.5		Increased
25	10	7		Increased
26	10	7.5		Increased
27	43	5	Increased	
28	43	5	Increased	
29	43	5	Increased	
30	35	5	Increased	
31	28	5	Increased	
32	14	6	Increased	
33	10	5.5	Increased	
34	10	5.5	Increased	
35	45	7	Increased	

DISCUSSION

In this study unique protein changes in cerium oxide treated cells have been observed through 2D gel electrophoresis. The 2D gel approach has allowed for the the identification of approximately 200 spots. But, it is important to disclose the limits of the of the analysis in that, the protein spots identified represent only a subset of the complete proteome. The protein spots identified only represent those proteins abundant enough to be visualized by silver staining. A cell holds approximately 25,000 protein coding genes, and with a number of these genes giving rise to splice variants. From this, it is clear that only a small percentage of the proteins expressed in this comparison are being viewed. A future direction would be to perform subcellular fraction to reduce the complexity in one total lysate and gain better coverage of the proteins present and expressed. This will provide a better dynamic range with enrichment of the proteins of interest.

The most significant conclusions that can be gathered from these results of the 2D gel electrophoresis, is that there is a noted increase in a small number of unique proteins identified in treated P19 cells when compared to the untreated P19 cells. These results imply that the majority of functional regulated systems are unchanged in the presence of cerium oxide exposure for 24 hours. However, this study does show the significant changes in the protein expression of several abundant proteins within 24 hours of cerium oxide exposure. Future experiments are required to perform mass spectrometry to reveal the protein populations that are visualized in this study. It will be important for the full identification of proteins found here to gain a better understanding of the proteins involved in the cells accommodations to being exposed to cerium oxide nanoparticles, for these proteins are controlled by transcription. A better understanding the genes being regulated with respect to protein will give an overall picture of how the cell is adjusting proteins involved in molecular, cellular component and biological processes, such as the cell cycle, transcription, translation, proliferation, mitochondrial biogenesis, and DNA repair just to name a few.

ACKNOWLEGEMENTS

We thank Dr. Michelle O. Claville Assistant Dean, Hampton University School of Science and Director of the NanoHU program for providing funding assistance in this research endeavor.

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