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Carbon Black and Titanium Dioxide Nanoparticles Differentially Activate Oxidative Stress and Apoptosis in A549 Human Alveolar Epithelial Cells

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Abstract

Recent studies have demonstrated that variation between particulate matter compositions have universally adverse effects on cells and living tissues. Carbon black and titanium dioxide are two such particulates that are continuously exposed to, yet there is limited research to examine the potential deleterious effects on living tissue. The objective of this study is to characterize the effect of carbon black (CB) and titanium dioxide (TiO2) on A549 human alveolar epithelial lung cells. CB and TiO2 powders were dispersed throughout a solution of water and bovine serum albumin by high- powered sonication. The effects of these particulates on A549 cells were analyzed through fluorescence microscopy, imaging, DAPI nuclear fluorescent staining, and western blotting, H2O2/DNA fluorescent staining.

Introduction

The endoplasmic reticulum (ER) is an organelle within eukaryotic cells that extends outward from the nucleus through the cytoplasm. It is the site of protein folding and modification, including glycosylation and formation of disulfide bonds, as well as the site of storage of free calcium, Ca2+ [1]. In the event of altered homeostasis of the ER lumens, proteins may stop folding correctly, which leads to the accumulation of unfolded proteins, and activates the unfolded protein response (UPR) [10]. There are three ER stress transmembrane proteins, ATF6, PERK, and IRE1, bound to ER chaperone protein 78 kDa glucose-regulated protein-binding immunoglobulin protein (GRP78/BIP), which activates the sensors when disassociating. When BiP dissociates, ATF6 is activated and will translocate to the Golgi, where it promotes release a cytosolic fragment that will co-activate transcription. PERK dimersizes and becomes autophosphorylated, and eventually autophosphorylates eIF2α, which eventually results in a halt in protein transcription. Inositol requiring enzyme 1a (IRE1α) becomes dimerized and trans-autophosphorylated, which then catalyses a 20-base intron out of X-box binding protein 1 (XBP1), which allows XBP1 to begin transcribing proteins needed in the UPR [11]. The IRE1α-XBP1 splicing mechanism is peculiar because RNA splicing is usually completed within the nucleus. If the UPR cannot correct the stress, a series of death cascades are activated ultimately resulting in apoptosis, programmed cell death.

Recent studies have demonstrated a link between cigarette smoke and particulate matter (PM) pollution in the air to the increase in cardiovascular related diseases and lung cancer. Exposure to cigarette smoke is associated with cellular oxidative stress, and cancer. Cigarette smoke consists of a mixture of gases and particulate matter including reactive oxygen species (ROS). Pollution particulate matter consists of meteor, salts, combustion material, and is primarily caused by traffic-related combustion. The molecular pathways through which nicotine and PM cause cytotoxicity and illnesses are not well understood [4,5]. It is known that PM causes increased levels of activated ATF6, GRP78/BIP, and phosphorylated eIF2α, but curiously, lower levels of spliced XBP1 [5]. It is also known that cultured cells exposed to cigarette smoke exhibit higher levels of phosphorylated eIF2α and GRP78/BIP, lower levels (and thus activation of) ATF6, and yet again, lower levels of spliced XBP1 [8].

It has been shown that cells, especially human lung epithelial cells, absorb small particulate matter, and ultrafine particles that can pass through the alveoli. However, the endoplasmic reticulum (ER) mechanism is still largely unknown [16]. Carbon black (CB) is mostly pure, ultrafine carbon particles which are emitted by combustion and industry, and it is known to be one of the main components of atmospheric pollution particles. Titanium dioxide (TiO2) is a naturally occurring mineral, but industries use it as ultrafine powder as a pigment in many cosmetic products such as sunscreen and toothpaste.

While our cells are exposed to cytotoxic ultrafine particulates on a daily basis, our cells have developed an advanced defense mechanism to ameliorate the stress induced by common toxic particles. CB and TiO2 NPs, a transcription factor in the cytosol, has been shown to be activated to a result of a nucleolar granules, which can be characterized as an accumulation of ROS in the cytosol, ER and mitochondria [9]. As a result of the accumulation of free radicals, NRF2 is activated by ROS directly and proceeds to translocate to the nucleus where it binds the ARE, antioxidant response element, and upregulates enzymes for scavenging ROS and general expression of essential glutathiones: HO-1, NADPH and GCL [2]. However, there have been contradictory findings in the activity of these NRF2 target genes. It is expected that the enzymes and antioxidants will be upregulated under particulate induced stress, however, there has been consistent evidence to support this theory. If HO-1, NADPH ad GCL cannot mitigate the cell’s redox dysfuntion, the apoptotic intrinsic pathway through the mitochondria is activated resulting in cell death [11].

The aim of this study is to characterize the effects of CB and TiO2 on A549 human alveolar lung cells and the particle’s ability to induce oxidative stress, caspase activation and apoptosis. Furthermore, we will be evaluating the cell’s defense response to the toxic particles by investigating NRF2 activation as well as the antioxidants and enzymes downstream of the redox transcription factor.

Results

To evaluate the oxidative stress induced by carbon black (CB) and titanium dioxide (TiO2), A549 cells were treated with CB (1 mg/ml) or TiO2 (1 mg/ml) in solution for 6 hours. To evaluate the mitochondrial stress induced by carbon black (CB) and titanium dioxide (TiO2), A549 cells were treated with 1 μg/ml CB or TiO2 for 6 hours. We detected the release of CB and TiO2 induced reactive oxygen species (ROS) with a DCFH-DA dye.

Conclusion

CB and TiO2 were dispersed in solution resulting in aggregates with size ranges beginning at 76 and 172 nm in diameter, respectively, through probe sonication. The nanoparticles are stable in solution as measured by zeta potential; CB: -31.63 ± TiO2: -10.85 ± (n=3). Carbon black treatment ranging from 50-100 μg/ml causes significant cell death after 24 and 36 hours whereas equivalent TiO2 concentrations do not.

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