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The Effects of Carbon Black on Cell Viability

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Abstract

Carbon black (CB) is a type of nanoparticle that is found in air pollution and is a known environmental toxin. The purpose of this work is to evaluate whether CB exposure activates cell death via apoptosis in cultured cell lines, supporting future work focused upon assessing the signaling pathways that might be induced by this exposure. Using adenocarcinomic human alveolar basal epithelial (A549) and baby hamster kidney (BHK-21) cells, we hypothesized that carbon black exposure causes cell death and potentially stress signaling via the endoplasmic reticulum (ER). The cells were exposed to CB and data collected for varied doses and time points. In order to measure cell apoptosis, the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method was used to detect apoptosis-associated DNA fragmentation. A 5 day exposure of CB at 100 ug/ml generated a significant reduction in cell survival and elevated numbers of TUNEL positive cells. Future work will focus upon assessing the stress pathways induced in these cells.

Introduction

Nanoparticles (NPs) are molecules that range from 1 to 100 nm in diameter in length [7]. They are made up of a variety of different elements such as silver, gold, titanium and carbon. Nanoparticles also have wide variety of applications in the biomedical field and personal products. One particular nanoparticle known as carbon black is a main component of air pollution. Carbon black is released into the air via automobile exhaust and power plant emissions. Alarmingly, there appears to be a link between pulmonary diseases and NPs; however the mechanisms at which this happens has not been thoroughly studied.

Several studies have also shown that certain NPs cause cytotoxicity. Some cytotoxic effects include oxidative stress and morphological changes in the cell. Oxidative stress is caused by an imbalance between reactive oxygen species (ROS) and antioxidant defense. ROS can cause damage in DNA, RNA and protein. Also, high levels of ROS can cause signaling pathways that lead to cell apoptosis. Ag is a NP that is found in many dental products. When human gingival fibroblast cells were exposed to Ag, the viability of the cell decreased and the generation of ROS increased [3]. Similar events occurred in bronchial cells when exposed to carbon black and titanium dioxide [1]. Cytoxicity can also induce morphological changes. Zinc oxide is a type of NP that causes nuclear shrinkage and chromatin condensation, both of which are also hallmarks of cell apoptosis [6].

The endoplasmic reticulum (ER) serves several different functions such as calcium storage, lipid metabolism and protein folding. ER stress is defined as anything that prevents the ER from functioning normally. In the event that the cell undergoes ER stress, the unfolding protein response (UPR) is activated. The UPR then continue on to imitate different signaling pathways that result acute, adaptive or apoptotic responses. Three different ER resident transmembrane proteins are involved in these signaling pathways. The proteins are: inositol requiring enzyme 1a (IRE1a), pancreatic ER eIF2a kinase (PERK), and activating transcription factor 6a (ATF6a) [5]. Under normal conditions, each of these proteins is inactivated and bound to a chaperone protein known as GRP78. When the cell experiences stress, the GRP78 is dissociated from these proteins. Once dissociated, PERK and IRE1a are activated through dimerization. These activated proteins then go on to activate other proteins to lead the cell onto the different pathways. ATF6 is a transcription factor that is translocated to the Golgi apparatus; where it is cleaved by proteasomes and then goes on to activate other proteins involved in the apoptotic pathway. [5].

My goal was to determine whether carbon black does indeed cause cell death. Previous studies have shown evidence that exposure to carbon black over a period of time causes morphological changes in the cell and nuclear condensation which are both potential indicators of cell death. For my experiment, I extended the time period in which the cells were exposed to carbon black and observed its effects on cell density as well as its effects on ER stress.

Results

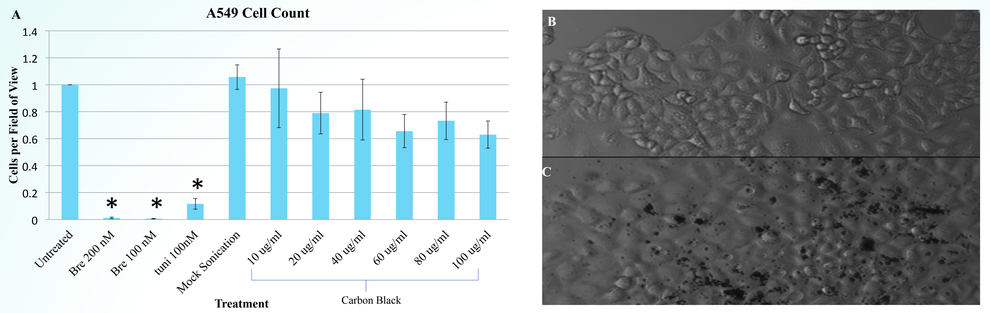


Figure 1. Quantification of A549 when exposed to CB. A549 cells were seeded at 10,000 cells/ml in 6 well plates. After 24 hrs of being seeded , the cells were treated with concentrations of CB that ranged from 10 to 100 ug/ml, as well as brefeldin-A and tunicamycin. Using the Nikon Eclipse TI microscope at 10x amplification, cells were counted each day for a total of 5 days. The morphology of the cell changed after 5 days of CB exposure. Small vacuoles were observed in some of the cells as well as infiltration of CB in the cell.

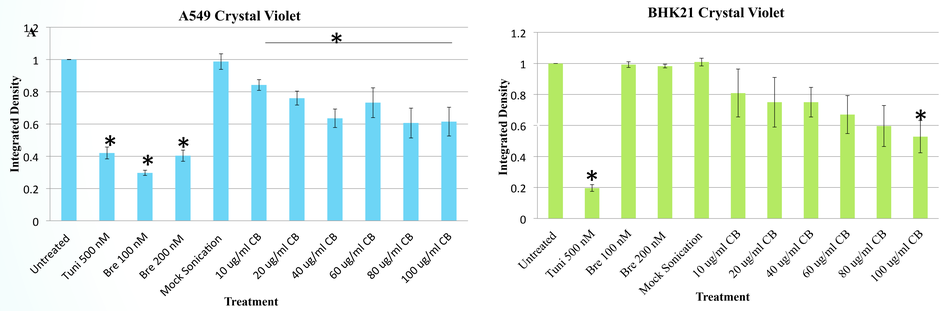
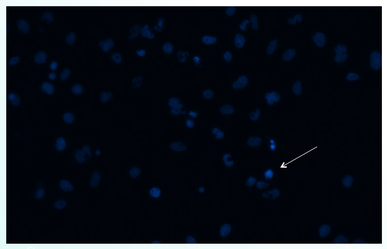
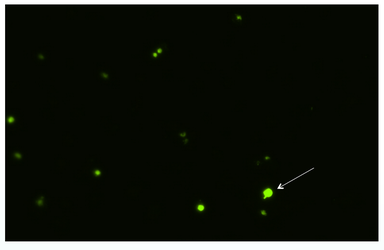
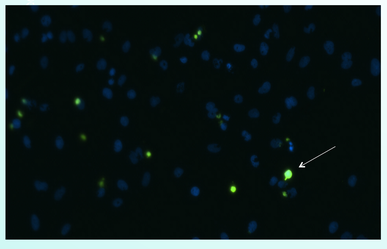


Figure 2.Crystal violet staining of BHK-21 and A549 after 5 days of treatment. After exposing the cells to brefeldin-A, tunicimycin and 10-100 ug/ml of CB for 5 days, the cells were fixed in 4% paraformaldehyde (PFA) and then stained with crystal violet four times, rinsed with dulbecco's phosphate buffered saline four times and lastly rinsed with deionized water. The cells were then left to dry for 20 minutes and cell density was analyzed with the Alpha Inotech.







Tunel Assay

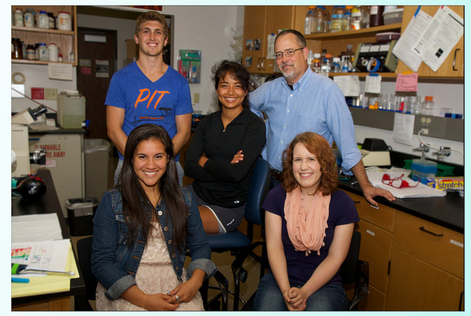
Cell Type	Treatment	% Apoptosis
BHK-21	Untreated	0.998
	Tuni 500 nM	57.559
	CB 30 ug/ml	3.174
	CB 60 ug/ml	6.519
A549	Untreated	0.341
	Tuni 500 nM	26.698
	CB 30 ug/ml	4.959
	CB 60 ug/ml	6.370

Figure 3. The TUNEL method was used to quantify apoptosis in BHK-21 and A549. The TUNEL method consists of terminal deoxynnucleotidyl (Tdt) labeleing 3' –OH DNA ends with a nucleotide that contains fluorescein labels. Apoptotic cells contain DNA fragmentation, and when analyzed under UV light, apoptotic cells will glow green. BHK-21 and A549 were seeded in 8 well chambers. After 24 hours they were treated with 500 nM of tunicamycin 30 and 60 ug/ml of CB for 24 hrs. The cells were then fixed with 4% PFA until the TUNEL experiment was carried out.

Conclusion

- Significant reduction in A549 with crystal violet when exposed to a range of 10-100 ug/ml CB.
- Significant reduction in BHK-21 when exposed to 100 CB ug/ml exposure.
- TUNEL method detected apoptosis in BHK-21 and A549.
- Future studies consist of assessing signal transduction induced by NP.

Acknowledgments



I would like to thank the National Science Foundation for providing funds for my research this summer. I would also like to express a special thank you to my mentor Dr. Brewster for helping me with my research throughout the summer as well as all of the faculty members and students participating in SURB.

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